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PENICILLIN LEVELS IN SERUM AND IN SOME BODY FLUIDS DURING SYSTEMIC AND LOCAL THERAPY

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CLARE WILCOX, AND MAXWELL FINLAND, M.D.
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THE rational choice of dosage and route of administration of penicillin presupposes some acquaintance with the effective concentrations of the antibiotic which can be attained and maintained at the site of the infection by the different methods. The desired concentration in any given case will depend, of course, on the susceptibility of the infecting organism. The variations in the sensitivity observed among different strains of some of the commonly encountered bacteria were considered elsewhere.[1] The present paper deals with the concentrations of penicillin obtained in serum and in certain body fluids of adult medical patients after administration of various doses of commercial penicillin given by different routes.

MATERIALS AND METHODS

Almost all of the observations included in this report were made on materials obtained during the course of treatment of infections on the medical wards. The penicillin was usually given in 0.85 per cent sodium chloride solution except in some of the cardiac patients, in whom continuous injections were given in 5 per cent dextrose solutions, and in those treated by inhalation. The concentration of penicillin in body fluids was determined by the serial dilution method of Rammelkamp.[2] A strain of hemolytic streptococcus No. 98, obtained from Dr. C. S. Keefer, was used throughout. Broth cultures seeded with approximately 10,000 of these streptococci per cubic centimeter were quite consistently sterilized by 0.0078 unit of commercial penicillin. In control tests in which the penicillin solution was contained in a volume of 0.2 c.c. and added to 0.5 c.c. of culture, the mixture was regularly sterilized by 0.0056 unit per cubic centimeter. Human Group 0 cells were used as an indicator in tests with body fluids in order to avoid the nonspecific hemolysis frequently observed when horse cells were used. Penicillin solutions for the standard controls were prepared at intervals from pools of five vials of commercial penicillin in sterile saline and stored at 5° C. The smallest amount of body fluid tested was usually 0.2 c.c. in a final volume of 0.7 c.c. of the culture. The smallest concentration measured in this manner was approximately 0.03 unit per cubic centimeter. End points were based on the smallest concentration of fluid in which there was no evidence of growth at eighteen hours and which yielded no growth in transplants on blood agar after another twenty-four hours. The method is subject to the wide limits of error common to such biologic tests.

SERUM LEVELS DURING CONTINUOUS INFUSIONS

Administration of penicillin by continuous infusions has the advantage of permitting the maintenance of fairly constant blood levels as well as avoiding repeated punctures. It does, however, require constant supervision. The intravenous route also necessitates the injection of larger volumes of fluid than

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are desirable in some of the types of cases in which it is best indicated and, in addition, has given rise to thrombophlebitis in a large percentage of the patients in whom injections were continued for more than forty-eight hours. The continuous intramuscular infusions are somewhat more difficult to regulate and are often quite painful, but they have proved somewhat more satisfactory in this clinic. Both methods are difficult to carry out properly in patients who are irrational and acutely ill. Neither method has been used routinely except when it was necessary to maintain high levels over a long period and when frequent injections were not well tolerated.

Serum levels under continuous infusion by either route did not usually exceed the average concentrations obtained during intermittent intramuscular injections of the same total daily dose, but given every two hours provided that a large fluid intake was avoided. Two examples may be cited from patients with bacterial endocarditis. In one of them, while receiving two-hour intramuscular injections of 15,000 units, the serum levels were 0.22, 0.22, 0.11, and 0.06 unit per cubic centimeter at intervals of one-fourth, one-half, one, and two hours, respectively, following the injections. During a long period of continuous intravenous infusion of the same total daily dose, the levels were usually 0.11 and only occasionally 0.22 unit per cubic centimeter. The second patient had serum levels of 0.22, 0.11, 0.11, and 0.06 unit per cubic centimeter at intervals of one-half, one, one and one-half, and two hours, respectively, after two-hour injections of 25,000 units, but the serum levels during continuous intravenous infusion of 300,000 units daily were repeatedly found to be only 0.06 unit per cubic centimeter. Thus, considerable variations were noted in different patients receiving intravenous infusions. In one instance, detectable levels could not be obtained at all during constant intravenous infusion of 100,000 units a day, while another maintained a serum level of 0.11 unit while receiving 6,000 units per hour intravenously.

Continuous intramuscular infusions also resulted in levels which were quite constant in any given patient but varied in different patients. For example, in one patient receiving 200,000 units daily for a cellulitis, successive serum levels were always 0.06 unit per cubic centimeter. A second patient who received 250,000 units a day for what proved to be miliary tuberculosis regularly had levels of 0.45 unit per cubic centimeter. A patient treated for subacute bacterial endocarditis with 300,000 units a day and tested on sixteen different days was found to have a level of 0.11 on eleven occasions, 0.22 on three, and 0.06 twice. In another similar case on the same dosage, a level of 0.11 was obtanied repeatedly.

Continuous Infusion in a Case of Anuria.—A patient who developed anuria during the course of sulfonamide therapy for pneumonia offered an opportunity for a study of the cumulative effect of continuous penicillin injection. He was given 100,000 units a day by a constant intravenous drip. The serum levels on four successive days after this was started were 0.22, 0.45, 0.90, and 1.8 units per cubic centimeter. Urine flow was re-established and penicillin was stopped soon after the last sample was obtained. There was no detectable penicillin (that is, less than 0.03 unit per cubic centimeter) in the serum obtained eighteen hours later.

SERUM LEVELS DURING INTERMITTENT INTRAMUSCULAR THERAPY

Penicillin concentrations in sera obtained at different intervals after intramuscular injections of 15,000, 20,000, and 25,000 units given at two-hour inter-

vals and after 15,000 or 20,000 units given at three-hour intervals are shown in Fig. 1. Most of these patients had good renal function and were not restricted with respect to their fluid intake but they took only moderate amounts. A few observations, specially designated in Fig. 1, were made on patients who had congestive heart failure. The latter patients were on a restricted fluid regime and had low urinary outputs.

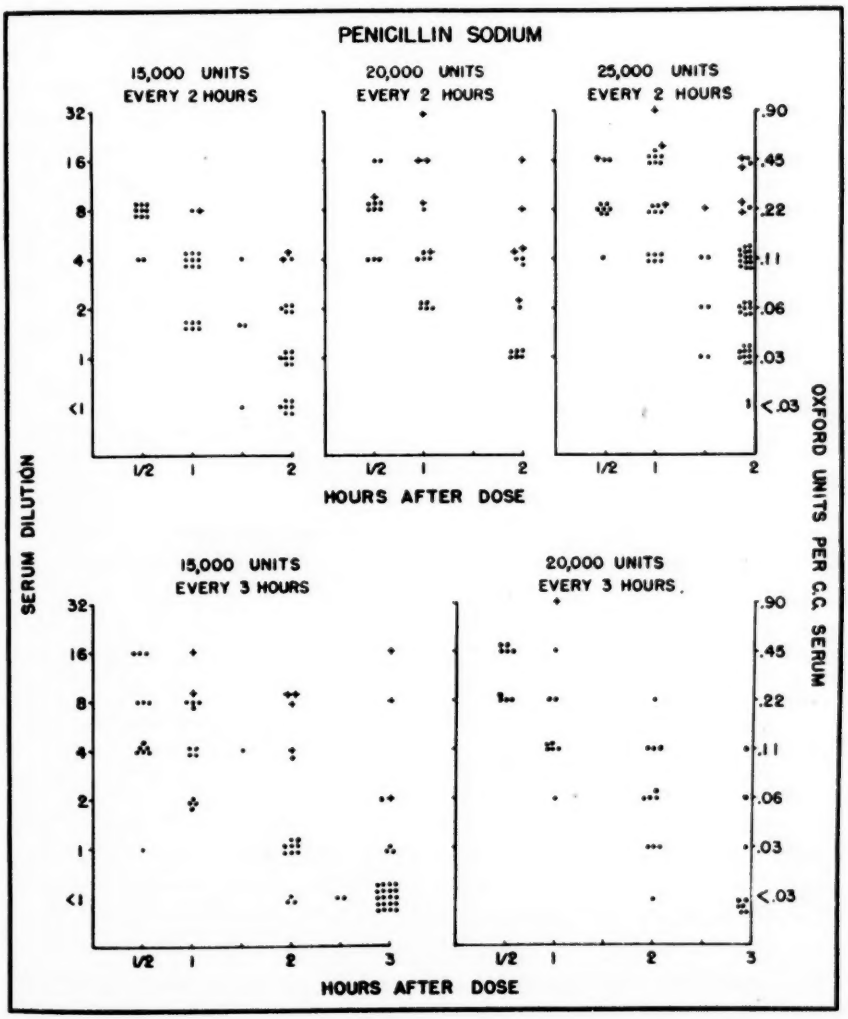
PENICILLIN SODIUM
15,000 UNITS EVERY 2 HOURS
20,000 UNITS EVERY 2 HOURS
25,000 UNITS EVERY 2 HOURS
15,000 UNITS EVERY 3 HOURS
20,000 UNITS EVERY 3 HOURS
SERUM DILUTION
OXFORD UNITS PER C.C. SERUM
HOURS AFTER DOSE
HOURS AFTER DOSE

Fig. 1.—Penicillin levels in the serum during intermittent intramuscular administration of commercial penicillin. The dots represent single observations in patients other than those with congestive cardiac failure. Observations in the latter are indicated by +.

In the patients without congestive cardiac failure it is seen that the levels obtained at any given interval after each dose fall within a fairly narrow range. The least variations were observed in the sera obtained at the one-half hour interval in patients who were being treated every two hours. The levels obtained at that time, moreover, were not much different with each of the dosage schedules that were studied in these cases. When 15,000 or 20,000 units were given every three hours, almost all of the sera obtained just before an injection failed to show detectable amounts of penicillin, and only an occasional one showed the smallest measurable amount, namely, 0.03 unit per cubic centimeter. Higher levels at one-half hour were more frequent after the 20,000 unit doses.

The greatest variations were noted in the sera obtained two hours after the injections. On the two-hour schedule about one-third of the sera obtained just before an injection of 15,000 units failed to show detectable amounts of penicillin; a similar number showed the smallest concentration measurable and the rest had twice that amount. With larger doses given every two hours, penicillin was found in almost every serum obtained before an injection. A greater proportion of the levels obtained at that time were higher on the 25,000 unit doses than on the lower amounts. The levels obtained one hour after the various doses also showed a general upward trend with increasing dosage.

Briefly, therefore, in febrile patients on a moderate but unrestricted fluid intake and with adequate renal function, there is less than 0.03 of a unit of penicillin per cubic centimeter of serum three hours after an intramuscular injection of 15,000 or 20,000 units. The same is true in one-third of the sera obtained two hours after the smaller dose. Larger doses give higher and more sustained levels.

In the patients with congestive cardiac failure the levels were uniformly more sustained and at significantly higher levels. The penicillin concentrations obtained in the serum three hours after a 15,000 unit injection in such patients were about comparable with the values obtained two hours after a 25,000 unit injection in ordinary cases.

PENICILLIN LEVELS IN CEREBROSPINAL FLUID

Some workers have failed to detect significant amounts of penicillin in cerebrospinal fluid after intravenous or intramuscular injections,[3-6] while others found appreciable concentrations, especially after very large doses in cases of meningitis.[7-10] In the absence of a block, diffusion of the penicillin usually takes place from the lumbar to the cerebral subarachnoid space and into the ventricles[3, 10] as well as in the opposite direction.[9] Penicillin has been detected in the spinal fluid for twenty-four hours and sometimes as long as ninety-six hours after injection by the lumbar route,[6, 10] but occasionally it cannot be detected even after fourteen hours.[9]

In the present studies cerebrospinal fluid was obtained by lumbar puncture in eighteen patients who had received one or more injections of penicillin intramuscularly. All but four of these patients had purulent meningitis. The lumbar puncture was done from one to two hours after an intramuscular dose of from 20,000 to 60,000 units, and venous blood was obtained at the same time. The levels of penicillin in the serum varied from 0.06 to 0.9 unit per cubic centimeter, more than half of them being 0.22 or 0.45 unit. Not one of the spinal fluids taken at the same time showed detectable levels of penicillin.

In thirteen patients under treatment for meningitis, concentrations of penicillin were also determined in spinal fluid obtained twelve or twenty-four hours after intraspinal injections of 10,000 or 15,000 units. The results are shown on Table I. The penicillin was given in a concentration of 1,000 units per cubic centimeter of saline. At twelve hours the levels in nine instances ranged from 10 to 40 units per cubic centimeter and, in one instance, 160 units per cubic centimeter were found. The levels at twenty-four hours ranged between 0.03 and 5.0 unit per cubic centimeter in eighteen instances, more than half of these being less than 1.0 unit. In one instance 80 units per cubic centimeter were found in the fluid at this time. At forty-eight hours, penicillin could not be detected in one case while intramuscular therapy was being continued.

In one patient who died twenty-four hours after a lumbar injection of 10,000 units, the fluid in the lumbar region contained 0.11 unit per cubic centi-

TABLE I. PENICILLIN LEVELS IN CEREBROSPINAL FLUID AFTER INTRASPINAL INJECTIONS

CASE	DOSE (UNITS)	INTERVAL (HOURS)	C. S. F. LEVEL (UNITS PER C.C.)
1	10,000	12	10.00
	10,000	12	40.00*
2	10,000	12	10.00
3	10,000	24	5.00
4	10,000	24	0.03
	10,000	24	0.11
5	15,000	12	40.00
	15,000	12	20.00
	15,000	12	160.00
	15,000	12	10.00
	15,000	24	80.00
	15,000	12	10.00
	15,000	24	5.00
	15,000	24	0.90
	15,000	24	0.06
	10,000	48	0.00
6	15,000	24	1.80
	15,000	12	10.00
	15,000	24	1.8
	15,000	24	0.22
	15,000	24	0.06
	15,000	24	0.90
7	15,000	24	0.22
		24	0.11†
8	15,000	12	20.00
9	15,000	24	2.5
10	15,000	24	0.06
11	15,000	24	2.5
12	15,000	24	1.8
13	15,000	24	0.22

*Ventricular fluid at this time had 0.9 unit per cubic centimeter.

†Obtained after death. No penicillin was detectable in the cisternal or ventricular fluid at this time.

meter and none was detected in the cisternal or ventricular fluid at autopsy. In a second patient who had a brain tumor, the spinal fluid twelve hours after an intraspinal injection of 10,000 units had 40 units per cubic centimeter and the ventricular fluid had 0.9 unit per cubic centimeter.

The reactions following intraspinal injections were similar to those noted by Rammelkamp and Keefer.[11] In three patients without meningitis receiving from 5,000 to 15,000 units one or two times, no symptoms resulted, and the only abnormality in the spinal fluid noted twenty-four hours later consisted in the appearance of from 23 to 138 lymphocytes per cubic millimeter without a significant increase in the protein content. In three similar cases, however, the injection of 15,000 units was followed by moderate to severe headache, malaise, and, in one instance, nausea and vomiting. The spinal fluid twenty-four hours later showed from 500 to 4,300 cells, mostly polymorphonuclears, and an increased protein content. The reactions in patients with meningitis who received intraspinal injections likewise varied. Some had an increase in the symptoms of meningeal irritation accompanied by a persistent or increased pleocytosis in the spinal fluid, and others showed return of the spinal fluid essentially to normal while daily injections were still being continued.

PENICILLIN LEVELS IN PLEURAL FLUIDS

Most workers have not found significant concentrations of penicillin in pleural fluids during intramuscular therapy.[12, 13] After intrapleural injections of from 5,000 to 70,000 units in patients with empyema, it is absorbed rather slowly so that considerable amounts have been found in the fluid up to ninety-six hours. The amount found and its persistence apparently depends on the

amount injected and on the nature and size of the cavity.[3, 12-17] Transient pleural pain, fever, and pleocytosis may result from injections into the noninflamed pleura.[14] Rammelkamp and Keefer[3] could detect no penicillin in the blood serum after an intrapleural injection of 5,000 units in one patient. They found only the smallest measurable amounts after 10,000 units in a second patient and up to 0.03 unit per cubic centimeter of serum after 30,000 units in a third patient in which samples were taken up to about seven and one-half hours.*

The present observations include (1) levels of penicillin in pleural fluid obtained after intramuscular injections alone, (2) serum penicillin levels after intrapleural injections when penicillin was not given by other routes, and (3)

TABLE II. CONCENTRATION OF PENICILLIN IN SERUM AFTER INTRAPLEURAL INJECTION

CASE	DOSE (UNITS)	INTERVAL (HOURS)	PENICILLIN IN SERUM (UNITS PER C.C.)†
1	50,000	1	0.06
		2	0.06
		3	0.06
		5	0.03
		12*	0.00
2	50,000	3	0.11
		4	0.03
		7*	0.00
3	50,000	1	0.00
		2	0.00
		3	0.00
		4	0.03
		5	0.03
		8*	0.00
4	20,000	1	0.00
		2	0.00
		3	0.00
		6	0.00
5	50,000	2	0.03
		4	0.03
		6	0.03
		8*	0.00
6	50,000	3	0.06
		5	0.06
		7	0.06
		9	0.03
		11*	0.00
7	50,000	2	0.11
		4	0.06
		6	0.03
		8*	0.00
8	50,000	4	0.11
9	50,000	5	0.03
10	50,000	1	0.22
		2	0.11
		4	0.03
		6	0.00
		8	0.03
11	60,000	1	0.11
		3	0.11
		6	0.11
12	60,000	1	0.03
		2	0.03
		4	0.03
		6*	0.00
13	50,000	½	0.22
		1	0.22
		2	0.11
14	40,000	4	0.22

Cases 6, 7, and 13 had serosanguineous effusions. The others all had empyema with thick purulent exudate, except Case 1, in which the fluid was serous in character.

*0 in one or more subsequent specimens of serum.

†0 here means <.03 unit which was the smallest amount measured.

*Florey and Heatley[25] obtained bacteriostatic levels in the blood stream for twenty-four hours after 120,000 units and for forty-eight hours after 240,000 units injected intrapleurally.

pleural fluid levels after intrapleural injections with or without concurrent intramuscular therapy.

Concentrations of penicillin in pleural fluids from one to two and one-half hours after intramuscular injections were measured in five patients with empyema and in one with rheumatic effusion. A single 40,000 unit dose was used on one patient, while the others were under treatment with doses of 15,000 or 20,000 units every two or three hours. Levels ranging from 0.03 to 0.22 unit per cubic centimeter of pleural fluid were obtained. The level in the serum taken at the time of the aspiration was the same in one instance and from two to eight times higher in the others.

Serum levels in fourteen patients at various intervals after intrapleural injections of from 20,000 to 60,000 units of penicillin are shown in Table II. The intrapleural penicillin was given in concentrations of from 500 to 1,000 units per cubic centimeter in saline, and none of the patients were receiving penicillin by other routes at the time. Penicillin was not detected in the serum in the one patient (Case 4) who received 20,000 units. In another patient (Case 3) in whom 50,000 units were injected, minimum levels were observed in the serum at four and five hours, but none was detected before or later. In the remaining patients, concentrations ranging from 0.03 to 0.22 unit per cubic centimeter were found in the serum up to five or six hours after the intrapleural injection of from 40,000 to 60,000 units. In only two instances were detectable amounts found later: after eight hours in Case 10 and after nine hours in Case 6. These levels are comparable with those obtained in the serum of different individuals about two hours after an intramuscular injection of 15,000 units as shown in Fig. 1.

TABLE III. CONCENTRATION OF PENICILLIN IN PLEURAL FLUID AFTER INTRAPLEURAL INJECTION

CASE	INTRAPLEURAL DOSE (UNITS)	INTERVAL AFTER DOSE (HOURS)	PENICILLIN IN PLEURAL FLUID (UNITS PER C.C.)
1	50,000	24*	5.00
		48	0.11
2	50,000	24*	0.03
	50,000	24	0.45
3	50,000	24*	2.5
		72	0.03
	50,000	24	0.45
	50,000	48	2.5
10	50,000	24*	0.11
	50,000	24	0.11
11	60,000	24*	2.5
		144	0.00
	50,000	48	5.00
12	60,000	24*	40.00
14	40,000	24	0.11
15	50,000	48	2.5
	50,000	48	1.8
	50,000	48	0.06
	50,000	48	0.22
16	10,000	24	0.9
	10,000	24	1.8
	10,000	24	1.8
		216	0.00
17	30,000	24	0.22
18	40,000	24	10.00
19	20,000	24	5.00
20	30,000	24	1.8

*These fluids were obtained after the injections listed on Table II.

Except in Case 20, in which there was a hydrothorax, all the fluids obtained before the injections were infected, thick, and purulent and were from patients with pneumococcal or streptococcal empyema.

Penicillin was given intramuscularly, usually 15,000 or 20,000 units every three hours, at the time these fluids were obtained, except Cases 2, 16, and 19, and before the aspirations indicated by the asterisks.

The concentration of penicillin in pleural fluids at various intervals after intrapleural injections of from 10,000 to 60,000 units varied considerably as shown in Table III. Intramuscular penicillin was being used in some and not in others. Even after the larger doses the concentrations in the pleural fluid obtained after twenty-four or forty-eight hours ranged from 0.03 to 40.0 units, most of them being between 0.11 and 5.0 unit per cubic centimeter. In some of the cases in which the concentrations in the pleural fluid were low, serum obtained at the same time showed higher levels. In one patient (Case 15), for example, serum levels obtained at the time of each of the last two fluids were 0.45 unit per cubic centimeter. In Case 10, the serum level each time was 0.22 unit per cubic centimeter.

Briefly, therefore, it would seem from the data presented that there is some diffusion from the blood stream into the pleural fluid as well as from the pleura into the blood, but it is quite variable in either case. Likewise, the concentrations found in the pleural fluid after the local instillation are erratic. This is probably due, at least in part, to the variable absorption from the pleural cavity. The possibility of loculation of infected fluids accounting for some of the wide discrepancies cannot be ruled out. There was no definite correlation between the specific gravity of the pleural fluid and the diffusion into it from the blood or from it into the blood. Furthermore, the concomitant intramuscular administration of penicillin did not seem to influence significantly the concentration in the pleural fluid after local instillation.

PENICILLIN LEVELS IN OTHER SEROUS CAVITIES

Very few observations have been reported on the diffusion in and out of body cavities other than the pleura and subarachnoid space. Small amounts have been measured in the serum for several hours after inoculation of 10,000 units into an infected knee joint.[3] This slow absorption from the synovial cavity has been suggested as a method of administering penicillin to obtain a prolonged systemic effect.[18] Penicillin has also been found in joint fluid during intravenous or intramuscular injections in a concentration about one-half of that found in the serum.[6] In infants and children, penicillin has been found in peritoneal fluid after intramuscular injection and also in the serum after intraperitoneal injections.[10*] The present study includes a few observations on synovial, peritoneal, and pericardial fluids.

Joint Fluids.—Six specimens were obtained by aspiration of the knee joints in three patients under treatment with 15,000 or 20,000 units of penicillin intramuscularly every three hours. Two of the patients had purulent gonococcal arthritis and one had acute rheumatic fever. The fluids were obtained from one-half to two and one-half hours after an intramuscular injection. The levels were 0.03 unit per cubic centimeter in five specimens and 0.06 unit per cubic centimeter in the sixth. These levels were the same or lower than corresponding serum levels.

Ascitic Fluid.—Infected peritoneal fluid was obtained on four occasions from one patient with cirrhosis of the liver and ascites who had a complicating bacteremic staphylococcal infection and was receiving 20,000 units of penicillin intramuscularly every two hours. The level of penicillin in the peritoneal fluid was 0.03 on two occasions, 0.06 on one, and none was detected in the fourth.

*Florey and Heatley[23] detected penicillin in the blood for thirteen hours in one patient and for twenty-four hours in another after injecting 120,000 units into an intact but infected knee joint. Greene and Alture-Werber[26] detected penicillin in the blood after intraperitoneal instillation.

One of the fluids showing 0.03 unit was obtained four hours after an intramuscular injection, and the serum had no demonstrable penicillin at that time. The sera obtained at the time of the two other positive fluids had the same concentration of penicillin as those fluids.

Pericardial Fluid.—Five specimens were available in two patients under treatment for purulent pericarditis. In one patient with streptococcal infection, 0.06 unit per cubic centimeter was found in the fluid aspirated during intramuscular therapy. In this case the fluids obtained twenty-four hours after each of two local injections of 20,000 units contained 2.5 and 5.0 units per cubic centimeter. The second patient had pneumococcal pericarditis and received two injections of 25,000 units each into the pericardial cavity after aspirations. The fluid obtained twenty-four hours later contained 20 units per cubic centimeter in one and 160 units per cubic centimeter in the other.

These findings indicate that penicillin diffuses into inflamed synovial, peritoneal, and pericardial cavities from the blood stream. From the pericardial cavity, as from the pleura and subarachnoid space, it seemed to diffuse out rather slowly in the two cases studied—at least high concentrations were still found twenty-four hours after the local injections.

SERUM LEVELS AFTER INHALATION OF PENICILLIN

The feasibility of utilizing penicillin in the form of an aerosol from a nebulized solution was pointed out by Bryson, Sansome, and Laskin.[19] These workers demonstrated penicillin in the lungs and urine of animals after inhalation. They were able to recover as much as 60 per cent of penicillin administered to cooperative human subjects who inhaled the aerosol directly from a nebulizer placed in the mouth. Clinical methods and apparatus for applying this procedure therapeutically were reported by Barach and his co-workers.[20] These authors demonstrated penicillin in the serum for one hour and occasionally longer after doses of from 40,000 to 100,000 units per cubic centimeter. They recovered from 10 to 20 per cent of the administered penicillin in the urine—most of this in the first hour.

The present observations were made during clinical trials of the methods described by Barach with some modifications by Dr. Maurice S. Segal, who also conducted the clinical studies. A total of eighty-one sera from seventeen patients under treatment with inhalations of from 20,000 to 30,000 units every two to four hours and from one normal subject after single inhalations of 20,000 or 25,000 units contained 1 c.c. of saline. Penicillin was not given by any other routes in these patients. The patients were being treated for acute or chronic pulmonary infections. The inhalations were usually completed within ten to twenty minutes. The frequency with which various levels were obtained at different intervals after the inhalations is summarized in Table IV. Significant

TABLE IV. PENICILLIN IN SERUM AFTER INHALATION

HOURS AFTER DOSE	PENICILLIN, UNITS PER C.C. OF SERUM					NUMBER OF SERA TESTED	PER CENT POSITIVE SERA*
	< .03	.03	.06	.11	.22		
¼		1	2			3	100
½	13	7	4	1	1	26	50
1	16	6	1	2	1	26	38
2	19	1	1		1	22	14
3	4					4	0
Total	52	15	8	3	3	81	35

*.03 unit per cubic centimeter or more.

The inhalations were given from nebulized solutions of from 20,000 to 30,000 units of commercial penicillin contained in 1 c.c. of physiologic saline every two to four hours and required from ten to twenty minutes to complete each dose.

concentrations were found in the serum for only a short time in most instances. In a few, however, penicillin was detected for as long as two hours. While most of the serum levels were low, occasional ones were comparable to those obtained two hours after intramuscular injections of 20,000 units in similar subjects. The highest and most sustained levels were obtained in two patients with pneumonia and in one with a lung abscess. Of the six sera showing levels of 0.11 unit or more per cubic centimeter, five were from a single patient who had a large lung abscess.

It should be borne in mind that these observations were made chiefly in sick patients, not all of whom could cooperate properly. Furthermore, the types of apparatus were being frequently changed and improved and relatively small doses were used. Higher levels can be expected for longer periods and more regularly with larger doses and improved apparatus in more cooperative patients.

DISCUSSION

It was the primary purpose of this paper to present observations on penicillin levels in serum and in some of the body fluids which are commonly infected with susceptible organisms. The data were obtained almost entirely on patients while they were under treatment. The method used is subject to considerable error, but it is adequate for these purposes since it was not intended here to offer any accurate measures of the absorption and fate of penicillin in the body.

Similar observations have been made with a different method by Selbie and co-workers[21] and with comparable results.

The concentrations of penicillin which destroy the common bacteria encountered in "medical" infections were studied in this laboratory by a very similar method[1] so that the results are comparable. They indicate that the smallest concentration of penicillin which was measured in the present study, namely, 0.03 unit per cubic centimeter, is adequate to sterilize actively growing cultures of almost all strains of gonococcus, group A hemolytic streptococcus, and pneumococcus and most strains of alpha hemolytic streptococcus, about half of the strains of meningococcus, and a somewhat smaller proportion of strains of pathogenic staphylococci. Undoubtedly the accessibility of the penicillin to the organism and many other factors, most of which are still unknown, also come into play and account for some of the discrepancies between in vitro and in vivo results.

The serum levels of penicillin obtained after various doses and intervals during systemic administration indicate that for any given dosage only a range of levels rather than any specific values can be predicted. In general, higher levels are attained and sustained with the larger and more frequent doses. The levels are not directly proportional to the increase in the dose except possibly in any given individual under constant intravenous therapy.[22] The results in patients with cardiac or renal insufficiency indicate that the volume of urinary output and the state of the kidney function may materially affect the height and persistence of penicillin levels after any given dose. Such patients usually attain and maintain any given level with appreciably smaller doses given at longer intervals than do persons with normal renal function and those who excrete larger volumes of urine.

The problem of whether or not topical injections are necessary in cases of meningitis or of infections of serous cavities or in walled-off focal abscesses will be answered more directly on the basis of clinical results than by any

analysis of data such as those given in this paper. The present observations and similar studies by others indicate that there is some diffusion into all serous cavities but that this is erratic and usually is only slight. Clinically, it is already recognized that these infections, after they have become established, do not often respond to systemic treatment alone in spite of the fact that bacteriostatic concentrations for the offending organisms can often be demonstrated in the exudate.

In cases of meningitis there are differences expressed in the literature both as to the penetration of penicillin into the cerebrospinal fluid and as to the necessity for having such penetration. Small amounts of penicillin have been found by others in individual cases particularly after massive systemic doses.[23] This has not been our experience nor that of others who have used larger doses in a greater number of cases. Some cases of bacterial meningitis have been reported as cured without resort to intrathecal injections of penicillin,[24] but these cases are not entirely convincing since few, if any, of these patients had received systemic penicillin as the only specific therapy. On the other hand, the experience in this clinic and in many others would indicate that, not only is intraspinal therapy necessary, but it is further necessary in some instances to resort to injections into the cerebral subarachnoid space or even into the ventricle[9] for complete recovery.

The necessity for systemic treatment when penicillin injections are given into infected body cavities will depend on the circumstances in each case. Some absorption takes place from all the cavities, but this, too, is erratic and effective levels are sustained in the circulation for variable periods. Some workers have found the poorest absorption from thick-walled cavities and from abscesses which are well walled off.[21] Obviously, therefore, if there is evidence of infection of tissues away from the infected cavity into which the penicillin is being injected, systemic treatment is definitely indicated. The absorption from the cavity may, however, be adequate to protect against spread of the infection from the site of the injection as a result of the procedure. It is possible that diffusion out of cavities is delayed in some cases when systemic therapy is given at the same time[10] and that may be a reason for using it. Evidence for this, however, is scant.

SUMMARY AND CONCLUSIONS

In this paper are presented the results of determinations of penicillin levels in serum and in some body fluids of patients under treatment with various doses of penicillin given by different routes. When compared with the results of tests for sensitivity of the offending organism, such information helps to indicate in a very general way the results to be expected from therapy in various types of cases.

During continuous intravenous or intramuscular administration, fairly constant levels are maintained in the serum of any given patient, but the levels may vary in different patients on the same dose.

During intermittent intramuscular injections a range of serum levels but not exact values can be predicted for the interval between doses on any given dosage schedule. Larger doses result in serum levels which are sustained longer and at higher values than smaller ones.

During intramuscular penicillin therapy it was not possible to detect penicillin in the cerebrospinal fluid, and the levels in pleural, peritoneal, and synovial fluids were erratic and usually lower than the serum levels.

Diffusion from local injections of penicillin into body cavities out into the serum is quite erratic, but adequate concentrations usually remain in cerebrospinal, pleural, and pericardial fluids for twenty-four hours and sometimes for forty-eight hours or longer after local injections of large amounts.

After inhalation of penicillin from nebulized solutions, small amounts may be detected in the serum for short periods but rarely for as long as two hours.

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RH AGGLUTINATION IN VARIOUS MEDIA WITH PARTICULAR REFERENCE TO THE VALUE OF ALBUMIN

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THE technique for the detection of anti-Rh antibodies has undergone several variations within the past few years. The first method in use, the "modified tube compatibility test" (incubation at 37° C. for one hour of unknown serum with 2 per cent suspension of Rh-positive red cells in saline), was found to be inadequate since from 40 to 50 per cent of the subjects in whom sensitization was later confirmed, by the birth of an erythroblastotic child or by a hemolytic transfusion reaction, showed negative tests for anti-Rh agglutinins. An explanation for such failures was found independently by Wiener and by Race. Race[1] found an additional evidence of Rh sensitization which he called an "incomplete antibody" that did combine with the antigen (the Rh-positive red cell) but failed to cause agglutination (in the saline suspension of cells in the test tube). At the same time Wiener[2] recognized the same phenomenon, that is, failure of agglutination of the Rh-positive cells by the serum of some sensitized individuals. He demonstrated it as follows: to the usual mixture of 2 per cent suspension of Rh-positive cells in saline and unknown serum from the sensitized person, he added one drop of an anti-Rh serum known to be capable of agglutinating the test cells. The difference in degree of agglutination of this system, after incubation at 37° C. and centrifugation, from the expected gross or 4 plus agglutination was the degree of inhibition or "blocking" caused by the unknown serum. Diamond and Abelson[3] have shown that the combination of these two anti-Rh tests (that is, ordinary agglutination plus "blocking" test) is much more satisfactory than the first alone, in that it demonstrated the presence of Rh antibody in 92 per cent of their cases of sensitization.

With the development of the rapid slide technique, Diamond and Abelson[4] proved that recognition of both agglutinin and inhibitor, or "blocking," antibodies was possible in almost 100 per cent of the individuals in whom sensitization had occurred. They found, however, that their test required Rh-positive cells in the form of whole blood and that the removal or marked dilution of the plasma of such blood (by washing repeatedly with saline) inhibited the reaction on the slide. As a result of these observations, they concluded[3] that plasma provided a medium in which Rh inhibitor, or "blocking," antibodies could cause agglutination or clumping of Rh-positive cells, and that absence or great dilution of this medium reduced the effect of the inhibitor antibody on test cells to merely a "blocking" action not demonstrable by any recognizable clumping. Wiener[5] suggested that this phenomenon might be due to a colloidal effect of the conglutinin of plasma. He named the reaction "conglutination" to distinguish it from agglutination in ordinary saline diluent. He confirmed that the results

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The use of albumin for enhancement of Rh agglutinins was suggested by the workers in the Department of Physical Chemistry, Harvard Medical School, and has been reported by Cameron and Diamond in the Journal of Clinical Investigation (in press).

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of the slide test could be reproduced in the test tube when plasma was substituted for saline as a diluent for the Rh-positive test cells.

Further studies have now been carried out on the effects of plasma in the testing of sera, which, though produced by sensitized individuals, failed to cause agglutination of Rh-positive red cells in saline diluent. Parallel titrations of a number of such known "blocked" sera, diluted serially in saline and in plasma, were set up, using twice-washed Rh-positive test cells likewise suspended in saline and in plasma. The technique was as follows: one drop of serum of each of the various dilutions was added to two drops of a 2 per cent suspension of the test cells in their respective diluent. The mixture was incubated at 37° C. for one hour, centrifuged at 500 r.p.m. for one minute, and read macroscopically with the aid of a hand lens. Weak and negative reactions were checked microscopically under low power. The titrations in the saline system showed no agglutination in any dilution, as previously had been demonstrated in these "blocked" sera. The plasma systems, however, showed strong clumping of the test cells. A typical result is shown in Table I. The

TABLE I. TITRATION OF ANTI-RH BLOCKING ANTIBODY IN SALINE AND IN PLASMA MEDIA (WASHED TEST CELLS, GROUP O, Rh_2; BLOCKING ANTIBODY, ANTI-Rh_o)

SERUM DILUTIONS	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256
2 per cent cells in saline (serum diluted with saline)	0	0	0	0	0	0	0	0	0
2 per cent cells in plasma (serum diluted with plasma)	Solid	Solid	++++	++++	+++	++	+	+	Trace

plasma diluent in this case either has supplied some factor by virtue of which the "blocking" effect of the antibody, as shown by the absence of reaction in saline, has been removed or has provided a more favorable medium than water, allowing the antibody-antigen reaction to become manifest by the clumping of the test cells to an end point of 1:256 with this particular serum. Thus the total content of Rh antibody is taken as 256, all of which is inhibitor, or "blocking," antibody. Similar experiments with sera containing both agglutinin and "blocking" antibody demonstrate that the saline diluent system, or, for that matter, any suitable salt in water, shows agglutination to one end point (often associated with a prozone phenomenon), whereas the plasma system has an end point at a much higher dilution. In such a case the ordinary agglutinin titer may be designated as the end point of the saline titration and the total antibody titer as the end point of the plasma titration, the difference between them being due to the "blocking" antibody present. A protocol of one of these experiments is shown in Table II. In this serum the ordinary agglutinin titer in saline is 1:8 and the total antibody titer, 1:512. The difference between these two levels is due to inhibitor antibody unmasked in the plasma diluent. Sera

TABLE II. TITRATION OF ANTI-RH BLOCKING-AGGLUTININ ANTIBODY IN SALINE AND IN PLASMA MEDIA
(WASHED TEST CELLS, GROUP O, Rh_1; ANTIBODY-AGGLUTININ, ANTI-Rh'; AND BLOCKING ANTI-Rh_o)

SERUM DILUTIONS	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
2 per cent cells in saline (serum diluted with saline)	++	+++	++	Trace	0	0	0	0	0	0	0
2 per cent cells in plasma (serum diluted with plasma)	Solid	Solid	Solid	Solid	++++	+++	++±	++	+	Trace	0

containing known agglutinin but no "blocking" antibody have been tested in this way and have shown the same end point of agglutination in both saline and plasma systems, indicating that all antibody present is demonstrated in the saline and that no further agglutination can be demonstrated by the use of plasma.

The use of plasma as a diluent for test cells, therefore, provides a method by which anti-Rh inhibitor antibody may be demonstrated in a test tube, the counterpart of the slide test of Diamond and Abelson. The exact nature of the mechanism by which plasma permits the demonstration of "blocking" antibody by means of agglutination will not be discussed here, although a colloid-like property, as suggested by Wiener, is undoubtedly one possibility. That complement is not responsible has been proved beyond doubt by the reproduction of the results described, using inactivated antibody serum and diluent plasma (heated at 60° C. for one hour). Dilution of the plasma with saline weakens or prevents the reaction of agglutination when the proportion of saline diluent to total contents of the tube reaches approximately one-third.* Once agglutination has occurred, however, the further addition of saline has no injurious effect.

Certain obstacles arose to the adoption of plasma as a diluent for test cells in anti-Rh determinations. Foremost among these was the unpredictable occurrence of rouleaux formaton in the test cells suspended in plasma; this appeared to be distinct from the clumping due to antibody-antigen reaction but nevertheless made gross and microscopic readings, especially of titration end points, often difficult to recognize. The cause of rouleaux formation could not be determined since it occurred in both Rh-positive and Rh-negative diluent plasmas, whether that removed from the cells themselves or that obtained from another source. In addition, the routine use of plasma demands a constant source of blood of Group AB (in order to avoid isohemagglutinin reactions if A or B test cells are used) and of the Rh- negative type (because the possibility of soluble Rh substance in plasma from an Rh-positive donor causing reduction or neutralization of Rh antibody in the serum being tested must be avoided). These objections to the use of plasma were of sufficient magnitude to make it desirable to seek a better suspension medium for test cells.

The following materials were tried as diluents for red cells: (1) albumin, human crystalline, (2) albumin, bovine crystalline, (3) gelatin, (4) methyl cellulose, (5) polyvinyl alcohol, and (6) dextrose (only because of its viscid nature). The anti-Rh sera for testing were selected because they had no demonstrable free agglutinin (against a saline suspension of test cells) but did have a high titer of "blocking," or inhibitor, antibody (demonstrated by the tube blocking test). The test cells consisted of twice-washed Group O Rh_1, Rh_2, and Rh-negative cells in 2 per cent suspension in each of the diluents. One drop of undiluted serum was placed in each of a number of small test tubes; two drops of the test cell suspensions in their respective diluents were added to each tube. The mixture was then shaken, incubated in a water bath at 37° C. for one hour, centrifuged at 500 r.p.m. for one minute, and read macroscopically. Negative reactions were confirmed microscopically. Parallel tests, using a saline and a plasma diluent, also were set up at the same time. The results with Rh_1 cells only (Rh_2 cells reacted in a similar way and, therefore, are not included in this protocol) are shown in Table III.

*This appears to be due not to the saline content, but to the water content. However, some electrolyte must be used in order to prevent hemolysis of cells.

TABLE III. THE ACTION OF ANTI-RH BLOCKING ANTIBODY ON RH + CELLS SUSPENDED IN VARIOUS MEDIA
(BLOCKING ANTIBODY, ANTI-Rh_o)

DILUENT MEDIUM FOR TEST CELLS	WASHED TEST CELLS	
	GROUP O, Rh_1	GROUP O, Rh NEGATIVE
Saline	0	0
Plasma	+++	0
Human albumin (25%)	+++±	0
Bovine albumin (30%)	++++	0
Gelatin	++++*	++±*
Methyl cellulose	+++*	+++*
Dextrose	0	0
Polyvinyl alcohol	0	0

*Matting and agglutination—cell damage.

The albumins, human and bovine, as suspension media for Rh-positive cells not only permitted clumping with "blocked" sera equal to that obtained with plasma, but in addition gave firmer and more distinct aggregations. Furthermore, the tendency to rouleaux formation was never encountered in the use of these materials. Reactions in bovine and in human albumin were equally satisfactory; as a result, the former was used in all of the succeeding experiments because of its greater availability and lower cost. Our experience with the use of albumin as a diluent has indicated that its greater density requires slightly more prolonged centrifugation in order to produce a satisfactory cell button at the bottom of the tube; 500 r.p.m. for two minutes has been found adequate.

No effect whatever was demonstrated by use of dextrose or polyvinyl alcohol. Gelatin and methyl cellulose caused a matting and clumping of Rh-positive and Rh-negative cells alike, although the clumping in 5 per cent gelatin was rather more intense with Rh-positive cells than with Rh-negative cells, which might be significant if other conditions such as pH, for example, were controlled. This substance, however, was extremely difficult to handle in view of its tendency to solidify at room temperature.

Attention was given next to the problems of optimum concentration of albumin, the effects of dilution upon its action with "blocked" anti-Rh sera and, in addition, the optimum concentration of test cells required. Dilutions of 30, 20, 15, 10, 5, and 2 per cent bovine albumin were made up. The upper level of 30 per cent was chosen because this is the maximum concentration of the commercial product available and above this the viscosity of the medium becomes objectionable. The technique of tube testing was that described plus the additional centrifuging. A typical result of these experiments is given in Table IV. It is apparent that albumin at a concentration of 10 per cent fails

TABLE IV. THE EFFECT OF VARYING CONCENTRATIONS OF ALBUMIN ON THE RH BLOCKING ANTIBODY-ANTIGEN REACTION
(BLOCKING ANTIBODY, ANTI-Rh_o)

CONCENTRATION OF BOVINE ALBUMIN DILUENT FOR TEST CELLS (%)	WASHED TEST CELLS		
	GROUP O, Rh_1	GROUP O, Rh_2	GROUP O, Rh NEGATIVE
30	+++±	++++	0
20	+++±	++++	0
15	++±	++++	0
10	0	++±	0
5	0	0	0
2	0	0	0

to permit clumping of Rh_1 test cells in this "blocking" serum, while a concentration of 15 per cent gives positive, though somewhat weak, reactions. Twenty per cent albumin, however, yields uniformly good reactions, and further experiments with many such "blocked" sera have corroborated this finding. When a cell diluent of 20 per cent albumin is used with this technique, it will be seen that the albumin content of the whole system in the tube falls to 13 per cent, ignoring the content of the original test serum. In repeated testing, this level of albumin concentration was found to be the critical point, below which reactions were weak or absent and above which they were uniformly strong.* The determination of optimum concentration of test cells in albumin was decided by the trial of various concentrations from 2 to 50 per cent of washed cells in 20 per cent albumin. All of these concentrations demonstrated equally good agglutination response to the antibody, although the 2 per cent suspension proved easier to prepare, handle, and read. For these reasons it was selected for use in the succeeding experiments.

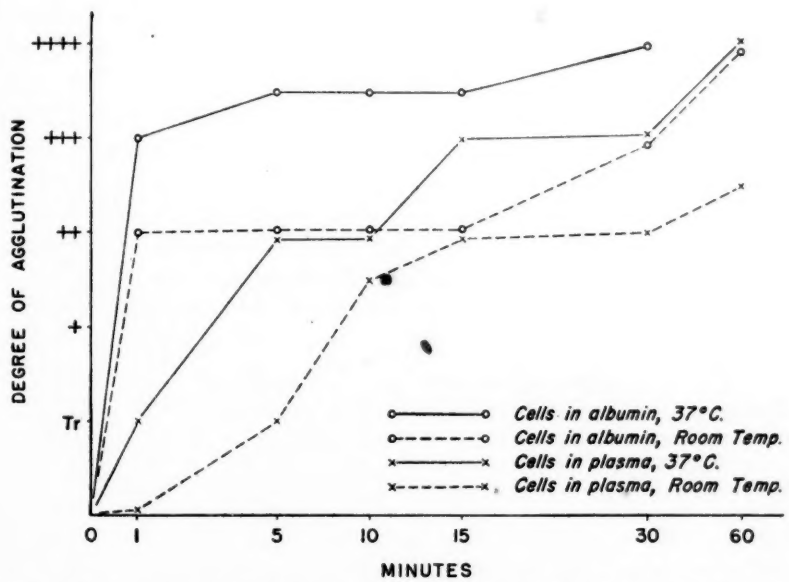

Fig. 1.—The rate of reaction of Rh-positive red cells in albumin and in plasma with Rh antibody of the "blocking" type.

The rate at which the antibody-antigen reaction takes place in plasma and in albumin was considered next. Two per cent suspensions of washed Rh_1 cells in plasma and in 20 per cent albumin were set up; one series of each was studied at 37° C., another at room temperature. The degree of reaction was read at one, five, ten, fifteen, thirty, and sixty minutes. The usual technique of incubation and centrifugation was used. The results of this experiment are shown in Fig. 1. Agglutination in the incubated albumin system was macroscopically visible in one minute, reaching completion between fifteen and thirty minutes. The albumin system at room temperature and the incubated plasma system showed similar rates of agglutination, reaching the stage of maximum agglutination between thirty and sixty minutes; the reaction in the plasma system at room temperature was still incomplete at sixty minutes. For routine technique, from

*The addition of albumin does not merely replace a deficit of albumin in the "blocking" serum, since albumin-globulin ratios in agglutinating and in "blocking" Rh antisera are within the normal range.

five to ten minutes in albumin and from fifteen to thirty minutes in plasma, at 37° C., yield valid and unmistakable results.

The effect of varying salt concentrations in the diluent media also was studied. Elliott[6] reported that anti-A and anti-B agglutination are enhanced by hypertonic sodium chloride concentrations in the test tube. Accordingly, Rh-positive cell suspensions were made up in three varieties of bovine albumin, hypotonic, isotonic, and hypertonic, and tested against a known "blocking" antibody. The hypotonic albumin system caused some swelling and hemolysis of test cells, and in addition the agglutinates were soft and difficult to read. On the other hand, the hypertonic and isotonic systems gave completely satisfactory results, the former causing slightly better agglutination than the latter.

A further experiment demonstrating that albumin is a satisfactory medium for the agglutination of Rh-positive cells by the inhibitor type of Rh antibody was carried out on a suggestion by Dr. Neva M. Abelson. Two drops of 2 per cent suspension of Rh-positive test cells in saline were "blocked" by the addition of one drop of antibody of a known inhibitor type. (Complete "blocking" was demonstrated by the tube method). The saline supernate was then withdrawn and replaced by two drops of 20 per cent bovine albumin. The mixture was shaken, allowed to stand at room temperature for five minutes, then centrifuged at 500 r.p.m. for two minutes, and read. Complete agglutination of the previously blocked cells had taken place, indicating that the presence of albumin had brought about a change in the test cells by which they were able to form agglutinates. This finding suggests a practical modification of the ordinary anti-Rh tests carried out in a tube with a saline suspension of test cells—if no agglutination of the saline suspension of test cells occurs (the reaction previously reported as negative), the supernatant saline is removed and replaced by albumin, the mixture allowed to stand at room temperature for five minutes, then it is re-centrifuged. The formation of agglutination in this new albumin system will prove the presence of inhibitor antibody in the unknown serum. Such a technique avoids the need for carrying out the tube "blocking" test, which requires the addition of another serum. Also it is economical in the amount of albumin used.

Having demonstrated the satisfactory elimination of "blocking" effect of Rh inhibitor antibody by the use of 2 per cent suspension of washed test cells in a diluent medium of 20 per cent albumin (human or bovine) of isotonic or slightly hypertonic salt concentration, a group of twenty Rh anti-sera, all containing inhibitor antibody, and some containing ordinary or early anti-Rh agglutinin as well, was studied by this technique. Each serum was tested with a 2 per cent suspension of washed Rh_1, Rh_2, and Rh-negative test cells in saline, plasma, and 20 per cent bovine albumin, respectively. In some cases additional tests were done with Rh′ and Rh″ cells. The results showed without exception that albumin and plasma diluents demonstrated, by agglutination of the test cells, the presence of antibody where it failed to react in the presence of saline diluent. Furthermore, the albumin suspensions gave stronger reactions than did plasma and were completely free from any rouleaux-like formation so frequently encountered in plasma. No reactions were seen which were not compatible with Wiener's classification of the Rh series.[7] Interpretation of the combined reactions of cell suspensions using red cells of different subtypes, in saline and in albumin made it possible to determine in every case the specificity of the inhibitor antibody and of the simple agglutinin (if present). In Table V is demonstrated a typical experiment by which the type of "blocking" antibody

TABLE V. THE DETERMINATION OF THE SPECIFICITY OF RH ANTIBODY (BLOCKING AND AGGLUTININ) BY THE REACTIONS OF TEST CELLS SUSPENDED IN SALINE AND IN ALBUMIN

(ANTIBODY-AGGLUTININ, ANTI-Rh′; AND BLOCKING, ANTI-Rh_o)

	WASHED TEST CELLS			
	Rh_1	Rh_2	Rh′	Rh NEGATIVE
2 per cent cells in saline	++++	0	++++	0
2 per cent cells in albumin	Solid	++++	Solid	0

may be specified. This serum shows agglutinin of type anti-Rh′, since agglutination Rh′ and RH_1 cells occurs in the saline suspension. However, agglutination of Rh_2 cells in the albumin system, but not in the saline, is evidence of the presence of a "blocking" antibody of specificity anti-Rh_o. This serum, therefore, is of the variety anti-Rh_o′ (87 per cent) with inhibitor of the Rh_o type. Such a serum is commonly used in routine laboratory testing (in saline suspension) as the 70 per cent or anti-Rh′ serum. "Blocking" antibodies in the group of sera tested were predominantly of the anti-Rh_o type, although two cases demonstrating inhibitor antibody of both Rh_o and Rh′ specificity were encountered.

Titrations of the three combinations of Rh antibody, i.e., simple agglutinin, combined "blocking" and agglutinin, and pure "blocking" antibody were carried out, the diluent media for test cells and antibody being saline and 20 per cent bovine albumin in each case. The results are shown in Table VI.

TABLE VI. TITRATION OF RH ANTIBODY OF THE VARIOUS TYPES WITH RH-POSITIVE CELLS, IN SALINE AND IN ALBUMIN SYSTEMS

SERUM DILUTIONS	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048
A. Agglutinin Only (WASHED TEST CELLS, GROUP O, Rh_2; ANTIBODY-AGGLUTININ, ANTI-Rh″, ONLY)												
2% cells in saline (serum diluted in saline)	Solid	++++	++++	++++	+++	+±	Trace	0	0			
2% cells in albumin (serum diluted in albumin)	Solid	Solid	++++	++++	++++	++±	++	Trace	0			
B. Agglutinin and Blocking (WASHED TEST CELLS, GROUP O, Rh_1; ANTIBODY-AGGLUTININ, ANTI-Rh′; AND BLOCKING, ANTI-Rh_o)												
2% cells in saline (serum diluted in saline)	++	+++	++	Trace	0	0	0	0	0	0	0	0
2% cells in albumin (serum diluted in albumin)	Solid	Solid	Solid	Solid	++++	++++	++++	++++	++	+	Trace	0
C. Blocking Only (WASHED TEST CELLS, GROUP O, Rh_1; BLOCKING ANTIBODY, ANTI-Rh_o)												
2% cells in saline (serum diluted in saline)	0	0	0	0	0	0	0	0	0			
2% cells in albumin (serum diluted in albumin)	Solid	Solid	++++	++++	++++	+++	+±	Trace	0			

From these findings it may be concluded that Rh-positive test cells in saline and in albumin diluents give the same type of reaction with the simple or early agglutinating antibodies; in the presence of inhibitor antibody alone the test cells in saline are "blocked," whereas in albumin they are agglutinated. When both types of antibody are present, their respective titers are indicated by an end point of agglutination in the saline system different from that in the albumin system. By such parallel titrations it is possible to determine the relative amounts of agglutinin and/or inhibitor antibody with considerable accuracy.

During these experiments a number of animal anti-Rh sera, produced by repeated injection of rhesus cells into guinea pigs, were titrated by the parallel saline and albumin medium method. One of these sera, previously rather unsatisfactory for routine use and readable only by the appearance of the cell button, showed a significant amount of inhibitor antibody, giving a markedly stronger reaction in albumin than in saline. A protocol of this experiment is shown in Table VII. It will be noted that the saline series shows a "prozone" effect which in our experience may be indicative of the presence of inhibitor antibody. This finding may explain the difficulties that have been encountered in the use of immune anti-Rh sera of animal origin. Such animal sera used with cells suspended in albumin may now yield entirely satisfactory results.

TABLE VII. COMPARATIVE TITRATIONS OF IMMUNE GUINEA PIG ANTI-RH ANTIBODY IN SALINE AND IN ALBUMIN MEDIA
(WASHED TEST CELLS, GROUP O, Rh_2; ANTIBODY, IMMUNE GUINEA PIG SERUM)

DILUTIONS OF SERUM	1:1	1:2	1:4	1:8	1:16	1:32	1:64
2% cells in saline (serum diluted in saline)	Trace	+	+±	+	Trace	0	0
2% cells in albumin (serum diluted in albumin)	Solid	++++	++++	++	+±	Trace	0

The following presents an interesting case illustrative of the value of albumin in permitting agglutination of Rh-positive cells by anti-Rh inhibitor antibody when saline suspensions of test cells had repeatedly yielded negative results.

The patient, a woman 60 years of age, had lost her last three infants with jaundice, anemia, and edema, the last (stillbirth) being thirty years previously. No further pregnancies or transfusions had occurred in the intervening years. Because of the particular interest of the patient and her physicians in proving serologically that Rh incompatibility had caused erythroblastosis and death of the infants many years ago, blood studies were carried out recently on several occasions. The patient's red cells were Rh negative, the serum showed no anti-Rh agglutinins when tested repeatedly against all subtypes of Rh-positive cells suspended in saline solutions. The tube test failed to identify "blocking" antibodies. The slide test, however, was positive, yielding a slow +± (1½ plus) reaction. Tests of the serum with washed Rh_1 and Rh_2 cells suspended in albumin showed definite 4 plus agglutination, and titration of the serum, diluted serially in albumin and tested against Rh_2 cells in albumin, showed an Rh antibody titer of 1:8, which was "blocking" antibody since a parallel titration in the saline diluent showed no reaction. The specificity of the inhibitor or "blocking" antibody was anti-Rh_0. This case demonstrates the sensitivity and certainty of results achieved by these newer testing methods for detection of Rh immunization. It also proves the long duration of permanence of such antibodies ("blocking" type); in this instance, thirty years post partum.

SUMMARY

The experiments described have followed a program of study of the action of anti-Rh antibodies, especially those designated as "blocking" or inhibitor antibodies, and their behavior toward Rh-positive cells suspended in various media. It has been recognized for a considerable time that certain types of Rh antibody, although known to be present, cannot be demonstrated readily by the tube agglutination of test cells suspended in saline, thus making the ordinary anti-Rh determination sometimes difficult and often inconclusive. The demon-

stration by Diamond and Abelson that these "blocking" sera would cause agglutination, on a slide, of Rh-positive whole blood provided the direction in which the experiments were aimed.

As reported elsewhere, in experiments involving the sensitization of Rh-negative subjects and in studies of sensitization of Rh-negative women by repeated pregnancies, it has been found that the simple anti-Rh agglutinin which produces clumping of Rh-positive cells in saline diluent is the first to appear and may therefore be called the early agglutinin or immature antibody. After repeated stimulation of experimental subjects or following multiple pregnancies in some women, inhibitor or "blocking" antibody appears. This may therefore be called the late, mature, or complete antibody.

Anti-Rh antibody, whether of the early, immature type or of the mature, late ("blocking") type, has been shown to react specifically with Rh-positive test cells in the test tube by the formation of clumps when a 2 per cent suspension of such cells in 20 per cent albumin is used. The degree of reaction, as manifest by the solidity of agglutinates, is considerably enhanced over that which occurs in saline suspensions, and in addition no inhibition of agglutination occurs when "blocking" or late antibody is present. Furthermore, serial dilution titrations of Rh antibodies regardless of the type (agglutinin, combined agglutinin and "blocking," pure "blocking"), using 20 per cent bovine albumin in one series and saline in another as diluent for both antibody and test cells, will demonstrate the relative amounts of agglutinin and of "blocking" antibody.

A modification of the tube anti-Rh test, involving the use of albumin in eliminating "blocking" effect, is proposed. This modification consists of the removal of supernatant saline in those anti-Rh tests which appear to be negative and replacement of this saline supernate by an equal amount of 20 per cent albumin. If any Rh antibody is present, agglutination of the previously non-reactive test cells will occur at room temperature within a few minutes.

The different behavior of Rh-positive cells of the various subtypes (Rh_o Rh′, Rh″) in saline and in albumin media toward Rh antibodies of the inhibitor type provides a method by which the specificity of such "blocking" antibodies may readily be determined. This technique is appreciably simpler and the results more clear-cut than the "blocking test" of Wiener.

No nonspecific effects of albumin have been encountered nor any behavior of Rh antibody with test cells not in keeping with the present classification of the Rh series. However, it should be kept in mind that if albumin is unavailable, plasma or serum which has been tested as free of nonspecific effects may be used for the tests described here.

As a result of these studies, as well as of observations during sensitization on the time relationship of the appearance of the simple agglutinating antibodies (active in saline suspensions of Rh-positive cells) and the "blocking" antibodies (inactive in saline but active in albumin), it seems advisable to consider more descriptive names for these two forms of the Rh antibody. The terms "early, or immature, antibody" would seem to apply to that causing agglutination in saline suspensions, whereas "late, or mature, antibody" is more descriptive of the "blocking" antibody which is recognizable only in albumin or plasma media and is associated with more severe or prolonged sensitization.

The use of albumin as a suspension medium for red cells has proved the potency of many sera previously discarded as containing little or no agglutinin and useless for laboratory determination of the Rh factor. Such sera may now become useful. This applies to some of the immune sera produced in guinea

pigs and other animals. A method for making many discarded sera valuable for routine use, even with saline suspension of red cells, is described in another communication.

Finally, as proved repeatedly in this laboratory and as described herewith in an illustrative case, an albumin suspension medium for test cells can readily detect weak agglutinins of the mature or "blocking" type in individuals who have been sensitized many years previously.

CONCLUSIONS

A series of experiments demonstrating the action of Rh antibodies on Rh-positive cells suspended in various media has been described. Inhibition of agglutination of Rh-positive cells by the so-called "blocking" antibody does not occur in the presence of plasma or albumin. Because of its freedom from non-specific effects, albumin has proved the most satisfactory suspension medium for test cells. Bovine acts similarly to human albumin and is, at present, considerably cheaper. It is important that the albumin content be added in a concentration above 13 per cent. The evaluation of early or immature antibody and of late or mature antibody is possible by comparative titrations in saline and in albumin systems.

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A CLINICAL EVALUATION OF THE COPPER SULFATE METHOD FOR MEASURING SPECIFIC GRAVITIES OF WHOLE BLOOD AND PLASMA

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A SIMPLE method for determining hemoglobin, hematocrit, and plasma protein values has recently been proposed by Phillips and co-workers.[1, 2] These values are derived from the specific gravities of whole blood and of plasma or serum with the assistance of line charts. The specific gravities are determined by the use of a series of copper sulfate solutions of graduated density which are readily prepared from a stock standard solution. The whole process requires no complicated apparatus and is easily adaptable to either laboratory or field work. The present study was undertaken to test the clinical applicability of this newly devised technique in a general hospital.

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Number of Tests for Which the Standard Copper Sulfate Solutions May Be Used.—Solutions of copper sulfate were prepared as directed by Phillips and associates in volumes of 50 c.c. with specific gravities ranging from 1.008 to 1.075, graduated in units of 0.002. The specific gravities within the range of 1.020 to 1.030 were measured by the gradient tube method of Linderstrøm-Lang[3] as applied by Lowry and Hastings[4] and showed a maximum deviation from the expected values of +0.0004 (Table I).

TABLE I. DETERMINATION, IN GRADIENT TUBE, OF THE SPECIFIC GRAVITIES OF COPPER SULFATE SOLUTIONS, BEFORE AND AFTER ADDITION OF 50 DROPS OF PLASMA TO 50 C.C. OF EACH SOLUTION

EXPECTED SPECIFIC GRAVITY	SPECIFIC GRAVITY FOUND BY GRADIENT TUBE		CHANGE CAUSED BY ADDITION OF PLASMA
	UNUSED SOLUTION	SOLUTION AFTER RECEIVING PLASMA	
1.0200	1.0204	1.0199	−0.0005
1.0220	1.0222	1.0219	−0.0003
1.0240	1.0242	1.0235	−0.0007
1.0260	1.0264	1.0256	−0.0008
1.0280	1.0283	1.0281	−0.0004
1.0300	1.0301	1.0299	−0.0002
Average change			−0.0005

A duplicate set of solutions, to each of which 50 drops of plasma had been added, was similarly checked by the gradient tube, and the average lowering of the specific gravity of the copper sulfate solutions, caused by adding one drop of plasma per cubic centimeter of solution, was found to be 0.0005 (Table I). Under ordinary conditions each set of 50 c.c. copper sulfate solutions can be used for determining the gravities of whole blood and plasma of about seventy-five blood samples without adding more than 50 drops to any one bottle.

Constancy of Results.—The first requisite of a quantitative method is that it yield results which can be duplicated within definite limits of error by different analysts. The copper sulfate method was tested in this respect by comparing duplicate specific gravities of the same specimens of whole blood and plasma determined by two observers (Table II).

Each specimen of blood in this work was drawn into a dry syringe. The greater part of the blood was delivered from the syringe into a hematocrit tube containing heparin. The excess blood left in the syringe was used at once to determine the gravity of the whole blood. The plasma separated in the centrifuged hematocrit tube was drawn into another dry syringe and used to determine plasma gravity.

From the gravities, plasma protein and blood hemoglobin concentrations were calculated by the following equations,[2] in which G_P represents plasma gravity and G_B the whole blood gravity. Equation 3 is derived from Equation 2 by substituting, for G_P, its mean normal value, 1.0264.

1. Gm. protein per 100 c.c. of plasma $= 389.6\ (G_P - 1.0079)$.
2. Gm. hemoglobin per 100 c.c. of blood $= 33.9\ \dfrac{G_B - G_P}{1.0970 - G_P}$
3. (Approximate hemoglobin from gravity of whole blood alone)
   Gm. hemoglobin per 100 c.c. of blood $= 480\ (G_B - 1.0264)$
4. Hematocrit (c.c. cells per 100 c.c. blood) $= \dfrac{100\ (G_B - G_P)}{1.0970 - G_P}$

These calculations can be made graphically by the line charts of Phillips and associates.[1, 2]

TABLE II. COMPARISON OF PLASMA AND BLOOD SPECIFIC GRAVITIES, AND THE CORRESPONDING PLASMA PROTEIN AND HEMOGLOBIN CONCENTRATIONS, AS DETERMINED WITH THE COPPER SULFATE METHOD BY TWO OBSERVERS ON THE SAME SPECIMENS (BLOOD SAMPLES DRAWN FROM UNSELECTED CASES ON MEDICAL WARDS)

CASE	SPECIFIC GRAVITY OF WHOLE BLOOD		SPECIFIC GRAVITY OF PLASMA		PLASMA PROTEINS (GM. PER 100 C.C. PLASMA)		HEMOGLOBIN (GM. PER 100 C.C. BLOOD)	
	ANALYST C	ANALYST D	ANALYST C	ANALYST D	ANALYST C	ANALYST D	ANALYST C	ANALYST D
1	1.0523	1.0520	1.0265	1.0265	7.23	7.23	12.42	12.25
2	1.0513	1.0510	1.0290	1.0295	8.21	8.40	11.12	10.80
3	1.0565	1.0560	1.0270	1.0265	7.43	7.23	14.28	14.18
4	1.0565	1.0565	1.0305	1.0303	8.79	8.71	13.24	13.25
5	1.0532	1.0530	1.0280	1.0280	7.81	7.81	12.38	12.28
6	1.0530	1.0530	1.0300	1.0305	8.59	8.79	11.64	11.47
7	1.0492	1.0498	1.0232	1.0235	5.96	6.07	11.94	12.14
8	1.0610	1.0600	1.0420	1.0428	13.28	13.59	11.72	10.77
9	1.0543	1.0540	1.0280	1.0277	7.81	7.70	12.92	12.86
10	1.0620	1.0620	1.0280	1.0278	7.81	7.74	16.72	16.76
11	1.0500	1.0497	1.0283	1.0277	7.93	7.70	10.55	10.76
12	1.0587	1.0593	1.0305	1.0308	8.80	8.90	14.37	14.49
13	1.0542	1.0545	1.0260	1.0258	7.04	6.97	13.46	13.66
14	1.0367	1.0365	1.0232	1.0235	5.96	6.07	6.19	6.00
15*	1.0545	1.0543	1.0271	1.0290	7.47	8.20	13.29	12.60
16	1.0651	1.0655	1.0288	1.0285	8.13	8.01	18.05	18.31
17	1.0558	1.0558	1.0268	1.0270	7.36	7.43	14.00	13.95
18	1.0577	1.0575	1.0310	1.0305	9.00	8.80	13.72	13.76
19	1.0582	1.0582	1.0280	1.0280	7.82	7.82	14.84	14.84
20	1.0585	1.0580	1.0290	1.0293	8.21	8.32	14.71	14.36
21	1.0435	1.0439	1.0252	1.0250	6.73	6.66	8.64	8.48
22	1.0475	1.0478	1.0290	1.0290	8.20	8.20	9.22	9.38
23	1.0620	1.0618	1.0301	1.0302	8.63	8.67	16.16	16.04
24	1.0522	1.0516	1.0273	1.0270	7.55	7.43	12.11	11.91
25	1.0565	1.0563	1.0270	1.0268	7.43	7.36	14.28	14.24
26	1.0552	1.0552	1.0272	1.0272	7.50	7.50	13.59	13.59
27	1.0528	1.0530	1.0260	1.0260	7.04	7.04	12.80	12.89
28	1.0495	1.0490	1.0275	1.0275	7.73	7.63	10.73	10.49
29	1.0603	1.0600	1.0281	1.0283	7.84	7.93	15.85	15.64
30	1.0588	1.0590	1.0282	1.0282	7.90	7.90	15.07	15.17
31	1.0520	1.0523	1.0277	1.0280	7.71	7.82	11.88	11.95
32	1.0590	1.0585	1.0280	1.0276	7.82	7.66	15.24	15.19
33	1.0585	1.0588	1.0287	1.0288	8.10	8.13	14.80	14.92
34	1.0421	1.0421	1.0198	1.0197	4.63	4.60	9.75	9.82
35	1.0443	1.0440	1.0270	1.0261	7.43	7.08	8.38	8.56
36	1.0587	1.0585	1.0272	1.0270	7.50	7.43	15.30	15.25
37	1.0582	1.0582	1.0282	1.0282	7.90	7.90	14.78	14.78
38	1.0605	1.0603	1.0275	1.0279	7.62	7.78	16.10	15.90
39	1.0523	1.0525	1.0260	1.0264	7.04	7.20	12.56	12.53
40	1.0505	1.0508	1.0254	1.0255	6.81	6.84	11.88	11.94
41	1.0491	1.0490	1.0230	1.0227	5.88	5.77	11.85	12.00
42	1.0642	1.0642	1.0283	1.0282	7.94	7.90	17.73	17.75
43	1.0568	1.0568	1.0255	1.0258	6.85	6.96	14.84	14.76
44	1.0507	1.0505	1.0301	1.0301	8.63	8.63	10.44	10.33
45	1.0411	1.0414	1.0228	1.0228	5.81	5.81	8.36	8.50
46	1.0377	1.0375	1.0210	1.0208	5.11	5.04	7.45	7.43
47	1.0515	1.0515	1.0255	1.0256	6.85	6.89	12.33	12.33
48	1.0580	1.0583	1.0275	1.0275	7.62	7.62	14.87	15.02
49	1.0584	1.0582	1.0273	1.0270	7.55	7.43	15.13	15.32
50	1.0572	1.0575	1.0249	1.0250	6.61	6.65	15.18	15.31
Mean difference	0.0003		0.0002		0.08		0.12	
Maximal difference	0.0010		0.0009		0.32		0.95	
96 per cent of duplicates are within	0.0006		0.0008		0.27		0.32	

*Results from Case 15 for plasma gravity, plasma proteins, and hemoglobin are excluded from calculation of mean, maximal, and 96 per cent differences because of obvious error in one of the plasma gravities in this case.

The agreement of results obtained by different analysts was close enough (Table II) to demonstrate that the copper sulfate method provides the required degree of constancy for clinical work.

Comparison of Results by the Copper Sulfate Method With Results by the Barbour Falling Drop Method.—The method of Barbour and Hamilton[5] has been in use for a number of years for estimating the specific gravity of plasma, and its accuracy has been satisfactorily demonstrated. In Table III results by this method applied to a number of plasmas are compared with results by the copper sulfate method. The agreement between the two is practically as close as the agreement between duplicates by either method.

TABLE III. COMPARISON OF PLASMA PROTEIN CONCENTRATIONS CALCULATED FROM GRAVITIES DETERMINED BY THE COPPER SULFATE METHOD AND THE BARBOUR FALLING DROP METHOD, RESPECTIVELY

	PLASMA PROTEINS (GM. PER 100 C.C.)*	
CASE	FROM GRAVITY BY $CuSO_4$ METHOD† a	FROM GRAVITY BY BARBOUR METHOD b
1	7.2	7.1
2	8.3	8.3
4	8.8	8.9
6	8.7	8.5
8	13.4	13.4
18	8.9	8.9
22	8.2	8.2
27	7.0	7.3
28	7.7	7.7
34	4.6	4.9
36	7.5	7.5
38	7.7	7.8
40	6.8	6.7

*Protein values from gravities by both methods are calculated as $Gm.\ protein\ per\ 100\ c.c. = 389.6\ (G_p - 1.0079)$.

†Mean of analysis *C* and *D*, Table II.

Comparison of Serum Protein Concentrations Calculated From Copper Sulfate Gravity Determination With Proteins Determined by Kjeldahl Analysis.—The results of this comparison are given in Table IV. The micro-Kjeldahl analyses were done with distillation and titration of the ammonia, the protein

TABLE IV. COMPARISON OF SERUM PROTEIN CONCENTRATIONS CALCULATED FROM SPECIFIC GRAVITIES BY THE COPPER SULFATE METHOD WITH CONCENTRATIONS DETERMINED BY MICRO-KJELDAHL ANALYSIS

	SERUM PROTEINS			OTHER BLOOD CONSTITUENTS	
CASE (TABLE II)	FROM GRAVITY BY $CuSO_4$ METHOD* (GM. PER 100 C.C.)	FROM KJELDAHL NITROGEN (GM. PER 100 C.C.)	DIFFERENCE, GRAVITY MINUS KJELDAHL (GM. PER 100 C.C.)	PLASMA GLOBULINS (GM. PER 100 C.C.)	BLOOD UREA NITROGEN (MG. PER 100 C.C.)
1	7.0	7.1	−0.1	4.9	5
2	8.1	7.4	+0.7	3.6	7
4	8.6	8.1	+0.5	–	36
6	8.5	8.0	+0.5	3.7	9
8	13.2	12.8	+0.4	9.2	5
18	8.7	9.0	−0.3	4.4	13
22	8.0	7.6	+0.4	3.5	6
27	6.8	7.0	−0.2	3.1	11
28	7.4	7.1	+0.3	3.3	10
34	4.5	5.3	−0.8	2.6	14
36	7.3	7.1	+0.2	4.0	6
38	7.5	7.0	+0.5	3.3	11
40	6.7	6.5	+0.2	4.5	5
44	8.4	8.6	−0.2	4.3	11
Mean difference			+0.15		

*The serum gravity was estimated at 0.0005 less than the mean of the duplicate plasma gravities of each case (Table II), in accordance with the statement of Phillips and co-workers, that the gravity of serum averages 0.0005 less than the gravity of plasma.

nitrogen being estimated as the difference between the total serum nitrogen and the nonprotein nitrogen and multiplied by 6.25 to calculate weight of protein. The nitrogen figures were available on serum, while the gravities were determined on plasma of the same blood specimens. However, the deviation of plasma gravity from serum gravity is so small that correction for the difference can be made with a fair degree of accuracy, as stated by Phillips and co-workers, by subtracting 0.0005 from plasma gravity to obtain serum gravity. From the serum gravities thus obtained the serum proteins were calculated by Equation 1.

In twelve of the fourteen cases, protein concentration estimated by gravity agreed within ± 0.5 Gm. per 100 c.c., with protein estimated by Kjeldahl; in the other two cases the differences, gravity minus Kjeldahl, were +0.7 and –0.8 Gm. The gravity method appears to be adequate for diagnosis of hyper- and hypoproteinemia.

Accuracy of Hemoglobin Values Estimated From Gravities of Whole Blood Alone.—For accurate estimation of hemoglobin from gravity values (Equation 2), it is necessary to use the gravities of both whole blood and plasma, since the hemoglobin calculation is based partly on the difference between these two values. However, as pointed out by Phillips and associates,[2] the effects of plasma gravity variations on the calculated hemoglobin values are usually small in comparison with the effect of whole blood gravity variations, so that in normal and most pathologic bloods approximate hemoglobin calculations can be made from whole blood gravity alone (by Equation 3) with the assumption that the plasma gravity has the mean normal value 1.0264. This procedure has the advantage that it enables one to make a bedside estimate of hemoglobin from finger blood. The results obtainable in clinical material are exemplified by the data in Table V. They show that in forty-seven of fifty cases the hemoglobin estimated from whole blood gravity alone was within 1.5 Gm. of the hemoglobin more exactly calculated from the gravities of both whole blood and plasma. In cases of hyperglobulinemia, however, such as may occur in multiple myeloma, plasma proteins may be so high that a gross error is caused in hemoglobin calculated by Equation 3. An example is Case 8, in which the plasma proteins were 12.8 Gm. per 100 c.c. (Table IV) instead of the assumed normal 7.2 Gm. The extra 5.6 Gm. of plasma protein caused a plus error of 4.7 Gm. in the hemoglobin estimated from whole blood gravity alone. Except in such gross hyperproteinemia, which appears to occur in about 1 per cent of patients on medical wards,[6] it appears that the whole blood gravity by itself may be used for approximate hemoglobin estimations, reliable within ±2 Gm. per 100 c.c.

Comparison of Hematocrit Values Calculated From Copper Sulfate Gravities With Hematocrit Values Determined by Centrifuge.—The results of this comparison are given in Table VI. Phillips and co-workers specify for hematocrit determinations centrifugation for sixty minutes at 3,000 r.p.m., with a radius of 18 cm. from the axis of the centrifuge to the center of the hematocrit tube. In the present analyses centrifugation was for the specified sixty minutes at 3,000 r.p.m., but the radius was shorter than 18 cm., so that the centrifugal force was less, and the cell columns obtained were somewhat longer. However, the ratio of column readings with the short-radius centrifuge to readings obtained with the standard 18 cm. radius was found to be constant and was applied to correct the observed readings. The centrifuge hematocrit values given in Table VI are those estimated for the standard conditions with a centrifuge of 18 cm. radius.

TABLE V. COMPARISON OF (a) HEMOGLOBIN CALCULATED FROM OBSERVED GRAVITIES OF WHOLE BLOOD AND PLASMA WITH (b) HEMOGLOBIN CALCULATED FROM OBSERVED GRAVITY OF WHOLE BLOOD ONLY, PLASMA GRAVITY BEING ASSUMED TO BE 1.0264 (GRAVITIES USED ARE MEANS OF C AND D, TABLE II; HEMOGLOBIN CALCULATED AS GRAMS PER 100 C.C. OF BLOOD)

CASE	HEMOGLOBIN CALCULATED		
	FROM G_B AND G_P BY EQUATION 2 a	FROM G_B ALONE BY EQUATION 3 b	DIFFERENCE b-a
1	12.34	12.49	+ 0.15
2	10.96	12.00	+ 1.04
3	14.23	14.47	+ 0.24
4	13.25	14.57	+ 1.32
5	12.33	12.92	+ 0.59
6	11.56	12.88	+ 1.32
7	12.04	11.18	− 0.86
8	11.75	16.50	+ 4.75
9	12.89	13.41	+ 0.52
10	16.74	17.23	+ 0.49
11	10.66	11.37	+ 0.71
12	14.43	15.77	+ 1.34
13	13.56	13.55	− 0.01
14	6.10	4.94	− 1.16
15	12.95	13.55	+ 0.60
16	18.18	18.83	+ 0.65
17	13.98	14.23	+ 0.25
18	13.74	15.10	+ 1.36
19	14.84	15.39	+ 0.55
20	14.54	15.44	+ 0.90
21	8.56	8.16	− 0.40
22	9.30	10.31	+ 1.01
23	16.10	17.18	+ 1.08
24	12.01	12.38	+ 0.37
25	14.26	14.52	+ 0.26
26	13.59	13.94	+ 0.35
27	12.85	12.82	− 0.03
28	10.61	11.08	+ 0.47
29	15.75	16.31	+ 0.61
30	15.12	15.73	+ 0.61
31	11.92	12.48	+ 0.56
32	15.22	15.68	+ 0.46
33	14.86	15.63	+ 0.77
34	9.79	7.60	− 2.19
35	8.47	8.62	+ 0.15
36	15.28	15.58	+ 0.30
37	14.78	15.38	+ 0.60
38	16.00	16.46	+ 0.46
39	12.55	12.58	+ 0.03
40	11.91	11.76	− 0.15
41	11.93	10.98	− 0.95
42	17.74	18.30	+ 0.56
43	14.80	14.71	− 0.09
44	10.38	11.72	+ 1.34
45	8.43	7.22	− 1.21
46	7.44	5.42	− 2.02
47	12.33	12.15	− 0.18
48	14.93	15.38	+ 0.45
49	15.23	15.44	+ 0.21
50	15.25	15.00	− 0.25

Summary.—Deviations of hemoglobin estimated approximately by gravity of whole blood alone, from hemoglobin calculated more exactly by gravities of both whole blood and plasma, were grouped as follows:

Deviations under 1 Gm., 37 cases (74 per cent)
Deviations under 1.5 Gm., 47 cases (94 per cent)
Deviations under 2.2 Gm., 49 cases (98 per cent)
Case 8 had gross hyperproteinemia, 12.8 Gm. protein per 100 c.c. plasma.

The agreement between gravity-estimated and centrifuged hematocrits in our pathologic bloods is less close than in the normal bloods reported by Phillips and associates; the standard deviation between the two sets of hematocrit values in our patients was ±2.6 c.c. of cells per 100 c.c. of blood, compared with ±1.2 in the normal bloods reported by Phillips and co-workers. The latter authors

point out that the gravity method is theoretically less accurate for hematocrits than for hemoglobin and that abnormality in erythrocyte specific gravity increases the error of gravity-estimated hematocrit values. Ashworth and Adams[7] found the mean deviation of gravity-calculated hematocrits from centrifuge hematocrits was three times as great in a series of pathologic bloods as in normal bloods.

In the series of fifty-three cases listed in Table VI, fifty-one show gravity-calculated hematocrit values, in cells per 100 c.c. of blood, that are within ±4.4

TABLE VI. COMPARISON OF HEMATOCRITS ESTIMATED BY THE COPPER SULFATE METHOD WITH HEMATOCRITS DETERMINED BY CENTRIFUGATION (FIFTY-THREE CASES)

CASE	$CUSO_4$ HEMATOCRIT a	CENTRIFUGE HEMATOCRIT b	DIFFERENCE a–b
A	31.0	29.6	+ 0.4
B	34.9	34.1	+ 0.8
C	42.5	39.4	+ 3.1
D	47.2	42.8	+ 4.4
E	45.5	42.8	+ 2.7
F	12.0	10.0	+ 2.0
G	28.0	25.4	+ 2.6
H	30.9	33.7	− 2.8
I	36.6	38.3	− 1.7
J	40.3	39.4	+ 0.9
K	21.2	22.8	− 1.6
L	38.0	39.2	− 1.2
M	36.1	33.3	+ 2.8
1	36.4	38.2	− 1.8
2	32.4	35.8	− 3.4
3	42.0	41.6	+ 0.4
4	39.1	39.9	− 0.8
5	36.4	37.8	− 1.4
6	34.1	35.1	− 1.0
7	35.5	36.6	− 1.1
8	34.7	31.4	+ 3.3
9	38.0	38.6	− 0.6
10	49.4	47.6	+ 1.8
11	31.5	33.2	− 1.7
12	42.6	45.7	− 3.1
13	40.0	39.5	+ 0.5
14	18.0	20.7	− 2.7
15	38.2	39.2	− 1.0
16	53.6	49.8	+ 3.8
17	41.3	40.6	+ 0.7
18	40.5	42.1	− 1.6
19	43.7	41.7	+ 2.0
20	43.0	43.6	− 0.6
21	25.5	25.1	+ 0.4
22	27.4	30.2	− 2.8
23	47.5	47.0	+ 0.5
24	35.4	36.1	− 0.7
25	42.1	41.6	+ 0.5
26	40.1	39.1	+ 1.0
27	38.2	40.5	− 2.3
28	31.3	30.0	+ 1.3
29	46.5	44.6	+ 1.9
30	44.6	35.2	+ 9.4
31	35.1	43.3	− 8.2
33	43.9	44.3	− 0.4
34	28.9	30.2	− 1.3
35	25.0	25.1	− 0.1
36	45.1	46.7	− 1.6
38	47.2	43.4	+ 3.8
39	37.0	39.6	− 2.6
40	35.3	35.3	0.0
41	35.2	36.4	− 1.2
42	52.3	50.6	+ 1.7
Mean difference			+ 0.06
Standard deviation from mean difference			± 2.6
Deviations under ± 4.0 c.c. in fifty of fifty-three cases.			

c.c. of the values determined by centrifuge and could be considered usable for ordinary clinical purposes. In the other two cases, however, the deviations were too great; namely, +9 and −8 c.c., respectively.

If the small proportion (4 per cent in our series) of patients with abnormalities like these two are excluded by preliminary comparisons of gravity and cen-

TABLE VII. COMPARISONS OF RED BLOOD CELL COUNTS AS DETERMINED BY DIRECT CHAMBER COUNT AND AS ESTIMATED FROM THE HEMOGLOBIN DETERMINED BY THE COPPER SULFATE METHOD; DUPLICATE DETERMINATIONS DONE BY DIFFERENT OBSERVERS (FIGURES FOR COUNTS REPRESENT MILLIONS OF CELLS PER CUBIC MILLIMETER)

CASE	R.B.C. CHAMBER COUNT			R.B.C. CALCULATED FROM $CuSO_4$ GRAVITIES			DEVIATION OF AVERAGE $CuSO_4$ R.B.C. FROM AVERAGE CHAMBER COUNT R.B.C.
	OBSERVER A OR C	OBSERVER B OR D	DIFFERENCE	OBSERVER A OR C	OBSERVER B OR D	DIFFERENCE	
			Normocytic Blood, (Twenty Specimens)				
A	4.9	4.9	0.0	5.4	5.2	0.2	+0.3
3	4.5	4.2	0.3	4.6	4.6	0.0	+0.25
4	4.0	3.9	0.1	4.3	4.3	0.0	+0.45
5	4.5	4.8	0.3	4.0	4.0	0.0	−0.65
7	3.7	3.4	0.3	3.9	3.9	0.0	+0.25
9	4.2	4.3	0.1	4.2	4.2	0.0	−0.05
10	–	5.8	–	5.4	5.4	0.0	−0.4
11	3.3	4.0	0.7	3.4	3.5	0.1	−0.2
13	4.9	4.9	0.0	4.4	4.4	0.0	−0.5
16	5.8	5.8	0.0	5.9	5.9	0.0	+0.1
17	4.9	4.4	0.5	4.5	4.5	0.0	−0.15
19	5.0	4.5	0.5	4.8	4.8	0.0	+0.05
20	3.9	4.2	0.3	4.8	4.7	0.1	+0.7
25	4.7	4.6	0.1	4.6	4.6	0.0	−0.05
27	4.6	4.2	0.4	4.2	4.2	0.0	+0.2
29	4.7	5.1	0.4	5.1	5.1	0.0	+0.2
33	4.8	4.6	0.2	4.8	4.9	0.1	+0.15
34	3.5	3.6	0.1	3.2	3.2	0.0	−0.25
44	3.4	3.7	0.3	3.4	3.4	0.0	−0.15
46	2.2	2.5	0.3	2.4	2.4	0.0	+0.05
Average difference			0.3			0.02	0.0
Spread			0.0 to 0.7			0.0 to 0.2	+0.7 to −0.65
			Hyperchromic Blood, (Fourteen Specimens)				
C	4.5	4.6	0.1	5.1	5.1	0.0	+0.55
D	4.5	4.9	0.4	5.0	5.0	0.0	+0.3
E	3.6	3.9	0.3	4.5	4.4	0.1	+0.7
F	3.9	3.4	0.5	3.9	3.9	0.0	+0.25
H	4.7	5.1	0.4	5.6	5.5	0.1	+0.65
I	3.0	3.1	0.1	3.5	3.6	0.1	+0.50
1	3.0	3.0	0.0	4.0	4.0	0.0	+1.0
6	3.3	3.1	0.2	3.8	3.7	0.1	+0.55
12	3.6	3.9	0.3	4.7	4.7	0.0	+0.95
14	1.5	1.4	0.1	2.0	1.9	0.1	+0.5
15	3.6	–	–	4.3	4.1	0.2	+0.6
21	2.7	–	–	2.8	2.8	0.0	+0.1
36	5.0	4.3	0.7	5.0	5.0	0.0	+0.35
40	3.2	3.7	0.5	3.9	3.9	0.0	+0.45
Average difference			0.3			0.04	+0.54
Spread			0.0 to 0.7			0.0 to 0.2	+0.1 to +1.0
			Hypochromic Blood, (Nine Specimens)				
8	4.7	4.1	0.6	3.8	3.5	0.3	−0.65
18	5.5	5.3	0.2	4.5	4.5	0.0	−0.9
22	3.9	4.0	0.1	3.0	3.0	0.0	−0.95
23	4.9	4.9	0.0	5.2	5.2	0.0	+0.3
24	–	4.1	–	3.9	3.9	0.0	−0.2
28	3.2	3.5	0.3	3.5	3.4	0.1	−0.1
35	3.5	3.6	0.1	2.7	2.8	0.1	−0.8
38	–	5.0	–	4.1	4.1	0.0	−0.9
Average			0.2			0.05	−0.55
Spread			0.0 to 0.6			0.0 to 0.3	+0.3 to −0.95

trifuge hematocrit values, it appears that in patients not thus excluded the gravity method can be used in following the subsequent clinical course of hematocrit changes.

Cell Counts Estimated From Gravities.—In bloods with a normal ratio of hemoglobin to cell count, the count can be calculated by Equation 5, in which the constant, 0.325, has been computed from data in the present paper.

$$5.\ \text{Million R.B.C. per mm.}^3 = \text{Hb} \times 0.325$$

where Hb. represents grams of hemoglobin per 100 c.c. of blood, estimated from copper sulfate determinations by Equation 2.

In Table VII is presented a comparison of values calculated by this equation with chamber counts on the same patients. Duplicate measurements were made by different observers. All bloods in the series with a color index between 0.9 and 1.1 were considered normocytic; no blood films were made.

Since in Equation 5 the assumption is made that the cells have mean normal values for both size and hemoglobin concentration, one would expect that hyperchromic bloods would give red cell counts which are too high, when calculated from Hb., and hypochromic bloods the converse. That this is true is seen from Table VII. Abnormality in the color index eliminates the usefulness of cell counts estimated from gravities.

The average difference between duplicate copper sulfate red cell counts is less than that between duplicate chamber counts. Results are therefore more exactly reproducible by the gravity method than by the chamber count.

Phillips and associates do not suggest estimating cell counts from gravities, but our results indicate that when a patient shows a normal color index (ratio of hemoglobin to cell count), the rapid gravity method can be used to estimate changes in cell count during treatment.

CONCLUSIONS

The copper sulfate method for specific gravities of whole blood and plasma, tested in the medical service of a general hospital, proved to be simple and accurate and to provide a quick procedure for estimation of hemoglobin, plasma protein, and, except in specific types of cases, hematocrit and cell count.

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THE THIAMINE REQUIREMENT IN ALLOXAN DIABETES

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PERIPHERAL neuritis is one of the frequent complications of diabetes. The factors underlying the development of this condition are as yet undetermined, but the possibility that thiamine may be involved has repeatedly been raised.[1-3] The known function of thiamine in the metabolism of carbohydrate,[4] together with its role in the development of certain forms of neuritis, has led some authors to believe that the requirement of this vitamin may be raised in diabetes and that the neuropathies are due to thiamine deficiency.[5] On the other hand, the diminished oxidation of carbohydrate which presumably takes place in diabetes would be expected to decrease the body's requirement. Alloxan diabetes offers a ready tool for the investigation of this problem. The following study was undertaken to determine if rats made diabetic by the injection of alloxan have a greater tendency to develop thiamine deficiency than normal controls and whether their requirement for thiamine is appreciably different from normal animals. A somewhat similar study has been reported by Styron and co-workers[6] using depancreatized rats, but the diabetes in their animals was very mild compared to the alloxan diabetes used in the following experiments, and no attempt was made by us to determine the effect of diabetes on the thiamine requirement.

EXPERIMENTAL

Adult male rats weighing between 250 and 350 grams were made diabetic by the subcutaneous injection of alloxan. A dose of 200 mg. per kilogram of body weight was given to the first ten animals, but nine of these died on the second day. Subsequently, the initial injections were reduced to one-half this dose. In animals resistant to this treatment, repeated daily injections gradually increasing up to 150 mg. per kilogram of body weight were made after a lapse of two or three days and were continued until the urine showed a consistent 4 plus reaction to Benedict's solution. Of thirty-nine rats treated, there were only eight fatalities by this technique, and none proved ultimately resistant though in one rat nineteen injections were necessary. There were no relapses from the diabetic to the normal state in this series. Following the injections of alloxan, all the animals suffered an immediate sharp fall in weight but over the course of the next week tended to maintain themselves at a new and, in all but one rat, lower level.

As soon as the diabetes in these animals was definitely established or shortly thereafter, they were divided into two groups. Group A consisted of seven rats which were placed on a thiamine-deficient diet composed of 74 per cent sucrose, 18 per cent casein, 4 per cent fat, and 4 per cent salt mixture.[7] Water-soluble vitamins were added to supply 4 mg. pyridoxine hydrochloride, 4 mg. riboflavin, 30 mg. nicotinic acid, 15 mg. calcium pantothenate, and 2.0 Gm. choline chloride per kilogram of ration.* One drop of haliver oil was given

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twice weekly to supply vitamins A and D. Group B consisted of four diabetic rats which were placed on the same diet plus 1 mg. of thiamine per 100 Gm. of ration. A control group, Group C, consisting of seven normal rats, was also given the thiamine-deficient diet. In the diabetic animals, frequent tests were performed with Benedict's solution to determine urinary sugar, and daily water consumption was measured. Weight changes were recorded daily.

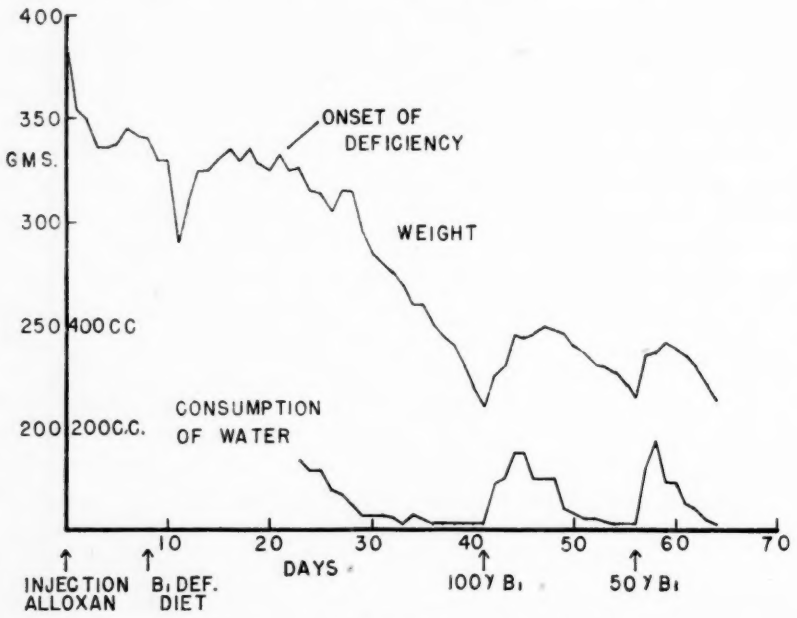

Fig. 1.—Thiamine deficiency in a typical diabetic animal and the response to therapy.

The time required to develop thiamine deficiency was taken as the time after the animals were given the deficient diet until weight loss became consistent. After the animals had lost from one-quarter to one-third of their body weight, the relative thiamine requirement of the diabetic animals versus that of the controls was determined by comparing the gain in weight following intraperitoneal injection of thiamine chloride. After a response to thiamine was obtained and the weight of the animals had fallen to the original level, another injection was given. Thus several responses, usually at different levels of thiamine, were obtained with each rat. In Fig. 1 is shown a typical example of the course followed by a diabetic animal.

RESULTS

The rats in Group B which received thiamine appear in good health after a period of four months although they are severely diabetic. They have demonstrated a marked increase in the consumption of both food and water, eating an average of 22 Gm. and drinking an average of 150 c.c. of water per day. Their weights may vary from 3 to 5 grams from day to day, and over a week up to 10 grams, but there has been no significant change following the initial period of stabilization. Their maintenance of weight indicates that marked and continued changes in the animals in Group A can be taken as an index of response to the administration of thiamine.

In Group A, diabetic animals on the thiamine-deficient diet, one rat died after two days. The others were continued on the ration, and after an average

of 16.1 days, developed listlessness and almost a complete loss of appetite and suffered a marked and continuous fall in weight. Water consumption gradually declined to from 2 to 5 c.c. per day, and urinary sugar dropped from a 4 plus to a 3 plus reaction. The normal control animals in Group C, receiving the same ration, developed similar symptoms and weight changes after an average of 14.1 days. After the death of one control, no further attempt was made to await the development of neuritis, but following the loss of not less than 80 grams of weight subsequent to the onset of signs of deficiency, both diabetic animals and controls were injected intraperitoneally with doses of 50, 100, or 200 μg of thiamine chloride. There followed an immediate alleviation of symptoms, a gain in weight, and in the diabetic animals a striking increase in the consumption of water and excretion of sugar. The maximum weight gain was found to be the most reliable index of response and the average results obtained at the different levels of thiamine administered are shown in Table I.

TABLE I. AVERAGE RESPONSE TO THIAMINE

THIAMINE DOSE (μG)	KIND OF ANIMALS	NUMBER OF TESTS	AVERAGE MAXIMUM GAIN (GM.)	PER CENT OF MEAN VALUE
50	Diabetic	6	27.8	108
	Control	5	24.0	93
100	Diabetic	7	39.4	116
	Control	4	28.5	84
200	Diabetic	3	46.0	116
	Control	2	33.0	84

The greater response of the diabetic animals at all levels of thiamine is apparently related to the composition of the material metabolized. During a period of four days, the food intake of the diabetic animals in Group B was measured. Sugar excretion[8] and urinary and fecal nitrogen were also measured quantitatively. Since these animals had maintained their weight over a prolonged period on this diet, the amount and composition of food actually metabolized was calculated as the sum of the fat eaten, the protein equivalent to the urinary nitrogen, and the carbohydrate eaten less the sugar excreted in the urine. The caloric requirement thus determined was approximately 40 calories per day, almost exactly the same as has been previously found by food consumption measurements on normal rats of the same weight.* However, to compensate for the continuous excretion of sugar, the diabetic animals ate approximately twice as much food as normal rats. Thus the composition of the diet actually metabolized was approximately 8 per cent fat and 36 per cent protein, compared to 4 per cent fat and 18 per cent protein in the controls.

DISCUSSION

The data from this experiment show that the tendency of the diabetic animals to develop thiamine deficiency was approximately the same or perhaps slightly less than the controls. That the thiamine requirement is actually less is convincingly shown by the average responses of the two groups to different levels of thiamine administration. At each level the response of the diabetic animals was greater than that of the controls. The data were analyzed statistically in one group by computing each response as per cent of a mean value midway between the two curves shown in Fig. 2. By the "t" test[9] the differences are highly significant (less than 1 in 500 that differences are due to chance alone).

*Unpublished data.

The somewhat greater average weight of the controls at the time thiamine was given does not appear to have been an important variable since no correlation between weight at the time of administration and subsequent response was found.

The lower thiamine requirement of the diabetic animals is logically supported by the data upon the composition of the diet metabolized. Both fat and protein have been shown to have a thiamine-sparing action.[10, 11] Wainio[11] has estimated that at a maximum, casein is 64 per cent glycogenic, and he employs a figure of 10 per cent for fat. Using these figures, it may be estimated that the thiamine requirement of the diabetic animals is approximately 12 per cent less than that of the control animals.

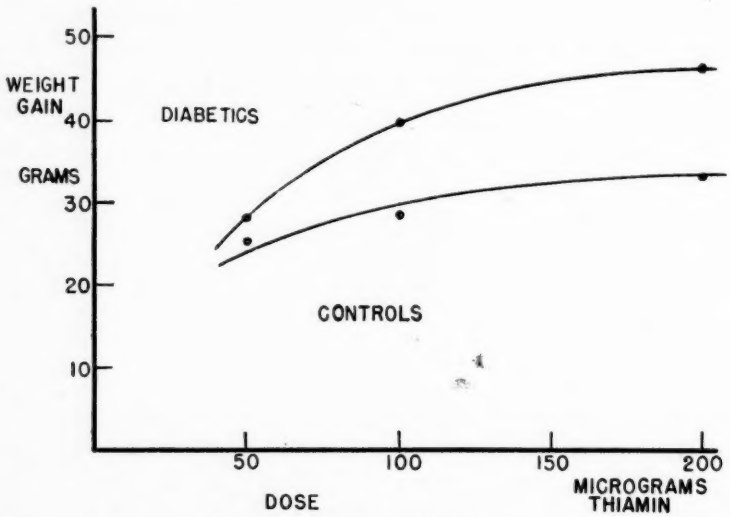

Fig. 2.—A comparison of the response obtained in diabetic versus control animals to graded levels of thiamine.

The findings in this experiment are of additional interest in view of the possibility that marked or prolonged diuresis may tend to "wash out" the body's stores of thiamine. Both prior to the onset of symptoms of deficiency and immediately following the administration of thiamine, the diabetic animals were drinking and excreting the equivalent in a 70 kilogram man of 30 liters of fluid per day. Furthermore, the injection of a single large dose such as was done here would particularly facilitate loss by this means. In view of the failure of these animals to develop deficiency before the controls and their normal response to thiamine administration, it is reasonable to conclude that either an appreciable amount of thiamine was not washed away or that their requirement is considerably less than indicated previously.

Caution must be used, however, in interpreting such experimental findings in terms of human patients. Alloxan diabetes obviously differs from that found in human beings, particularly with regard to capacity to survive without protection by insulin. Furthermore, Rudy and Epstein[5] have reported partial or complete relief of neuritis in 67 of 100 diabetic patients by the administration of supplementary thiamine. However, the exact basis for this therapeutic response is not clear. The role that diet may play in the pathogenesis of this condition is in dispute. Jordan,[12] reviewing 226 cases at Joslin's clinic, concluded that diet was not a factor, while Rudy and Epstein believe that it cannot be ruled out in 73 per cent of their patients. The latter authors have not, however, indicated the method of dietary analysis by which they reach this conclusion. In addition, the possibility remains that there may be a disturbance in thiamine

utilization in human beings with diabetes, although there is no evidence available to support such a hypothesis at this time.

CONCLUSION

This study presents evidence that rats made diabetic by injection with alloxan show no increased tendency to develop signs of thiamine deficiency, that the action of thiamine is not perceptibly impaired, and that the diabetic animals' requirement of this vitamin is less than that of normal controls. It does not support the theory that diabetic neuritis is due to thiamine deficiency.

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BACTERIOSTATIC PROPERTIES OF SULFANILAMIDE AND SOME OF ITS DERIVATIVES

III. The Carboxy-Sulfathiazoles as Chemotherapeutic Agents Active in the Gastrointestinal Tract

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SUCCINYLSULFATHIAZOLE (Sulfasuxidine) was introduced as an intestinal antiseptic by Poth and Knotts in 1941.[1] In 1943, Poth and Ross[2, 3] published the results of a continued study of these dibasic acylated sulfonamides and presented data on phthalylsulfathiazole (Sulfathalidine); this drug was shown to possess approximately twice the bacteriostatic activity of sulfasuxidine when the antibacterial activty was measured against the coliform organisms in the gastrointestinal tract of man and dog. Sulfasuxidine has had extensive

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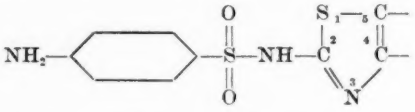
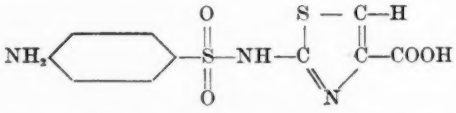
clinical trial[4-23] and has become well established as a useful drug, being particularly valuable in the treatment of bacillary dysentery and in the preoperative preparation of the bowel. While sulfathalidine has had a less extensive clinical use,[4, 22, 24, 25] it has been shown to be especially valuable in controlling nonspecific diarrheas, chronic ulcerative colitis,[26] and regional enteritis and in the preoperative preparation and postoperative treatment of the bowel. Both of these compounds are finding extensive use in the treatment of chronic urinary tract infections due to the coliform organisms.[24, 27] These two compounds are poorly absorbed from the gastrointestinal tract; this properly would not be anticipated since the molecules contain a free carboxyl group and readily form ionizable salts. The presence of a free carboxyl group has, however, led to the study of another series of sulfonamides, the carboxylsulfathiazoles, where the substitutions were on the 4 and 5 carbon atoms of the thiazole rest.



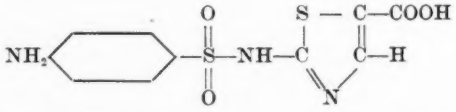
The carboxysulfathiazoles studied were* (Table I):

1. Sulfa-4, carboxythiazole



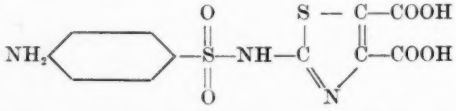
(Mol. wt., 299.3; M. P., 240-241° C. with decomposition; relatively stable chemically)

2. Sulfa-5, carboxythiazole



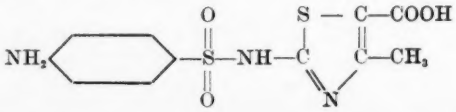
(Mol. wt., 299.3; M. P., 210-215° C. with decomposition; relatively unstable chemically and yields sulfathiazole by splitting out CO_2)

3. Sulfa-4,5, dicarboxythiazole



(Mol. wt., 343.2; M. P., 240° C. with decomposition; will not form acid salts and will decompose to yield sulfa-4, carboxythiazole)

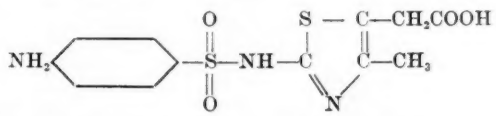
4. Sulfa-4, methyl, 5, carboxythiazole



(Mol. wt., 313.3; M. P., 190-200° C. with decomposition; relatively unstable chemically and will yield sulfa-4, methylthiazole)

*These compounds were supplied by Sharp and Dohme Inc., Philadelphia, Pa. The authors are indebted to Dr. J. M. Sprague for the chemical data and physical constants presented.

5. Sulfa-4, methyl, 5, carboxymethylthiazole



(Mol. wt., 327.3; relatively stable chemically)

These compounds were studied in a manner similar to that of the acylated sulfonamides previously reported.[5, 22] The antibacterial activities of these chemicals, as indicated by the alteration of the coliform organisms in the gastrointestinal tract, were determined by administering them to dogs at four-hour intervals throughout the day and night. The animals were placed on a submaintenance diet of boiled horse meat. The drug was mixed with this food, which was made into balls, and fed automatically at four-hour intervals by a device described by Poth.[28] Since the animals always were hungry, they ate their food as soon as it became available so that the drug was ingested regularly.

Stool specimens for bacteriologic study were taken directly from the rectum. The number of coliform organisms was determined by plate counts on desoxycholate media containing 5 mg. per cent of p-aminobenzoic acid.

Blood and urine were analyzed for sulfonamide by modifications of the usual colorimetric procedure. The urine was obtained directly from the bladder by catheter.

The results of these studies are summarized in Table I and Figs. 1, 2, 3.

TABLE I. THE LOCAL ANTIBACTERIAL ACTIVITY OF THE CARBOXYTHIAZOLES IN THE GASTROINTESTINAL TRACT OF THE DOG, WITH THE DOSAGE, MAXIMUM CONCENTRATIONS OF THE DRUGS IN THE BLOOD AND URINE, AND THE RELATIVE TOXICITY OF THE SEVERAL COMPOUNDS

DRUG	DAILY DOSAGE* (GM. PER KG.)	MAXIMUM CONCENTRATION OF DRUG IN BLOOD (MG. %)	MAXIMUM CONCENTRATION OF DRUG IN URINE (MG. %)	RELATIVE ANTIBACTERIAL ACTIVITY	RELATIVE TOXICITY
Sulfa-4-carboxythiazole†	0.25	---	----	++	0
	1.0	6.0	1450	+++	0
Sulfa-5-carboxythiazole†	0.25	---	----	++	0
	1.0	6.5	2200	++++	0
Sulfa-4,5-dicarboxythiazole†	0.25	---	----	+++	0
	0.50	6.2‡	335‡	++++	0
	1.0	6.8‡	590‡	++++	0
Sulfa-4-methyl-5-carboxythiazole	0.5	10.0	----	++	0
	1.0	18.0	1800	+++	+++
Sulfa-4-methyl-5-carboxymethylthiazole	0.5	6.2	552	+++	0
	1.0	6.2	554	+++	0

*The daily dose was divided into six equal portions and administered at four-hour intervals.

†Two intravenous doses of 1.0 Gm. each given at twenty-four hour intervals. Drug dissolved by addition of equivalent quantities of NaOH. No toxicity demonstrable.

‡These values are inaccurate because an entirely satisfactory analytical procedure could not be devised.

In Vitro Activity.—Doctor W. F. Verwey and co-workers observed the in vitro antibacterial activity of the carboxysulfathiazoles as follows: A 1:10,000 dilution of a twenty-hour culture of *Escherichia coli* was cultured in a synthetic medium containing varying amounts of the sulfonamide and taking as the end

point that molar concentration which allowed a growth giving a turbidity equal to 50 per cent of that of the control tube when incubated for twenty hours.

Sulfathiazole	4×10^{-7}
Sulfanilamide	1×10^{-4}
Succinylsulfathiazole	7×10^{-4}
Sulfa-5-carboxythiazole	8×10^{-5}
Sulfa-4-carboxythiazole	6×10^{-4}
Sulfa-4,5-dicarboxythiazole	3×10^{-3}
Sulfa-4-methyl-5-carboxymethylthiazole	1×10^{-3}

Comparison of the bacteriostatic activity of these compounds in the intestinal tract, as shown in Table I, with the in vitro antibacterial activity, fails, as is so frequently true, to show any correlation.

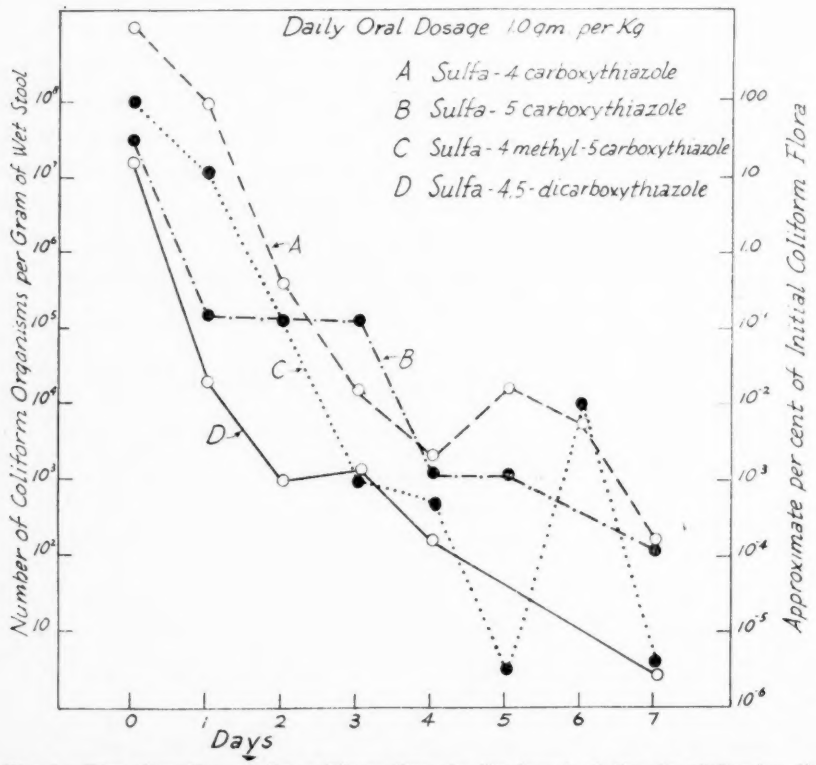

Daily Oral Dosage 1.0 gm. per Kg
A Sulfa-4 carboxythiazole
B Sulfa-5 carboxythiazole
C Sulfa-4 methyl-5 carboxythiazole
D Sulfa-4,5-dicarboxythiazole
Number of Coliform Organisms per Gram of Wet Stool
Approximate per cent of Initial Coliform Flora
Days

Fig. 1.—The alteration of the coliform flora in the bowel of the dog following the oral administration of carboxysulfathiazoles. At this dosage level, the alterations are identical within the limits of experimental error. The population of these organisms is decreased to 1/100,000,000 of the original.

Sulfa-4,5-Dicarboxythiazole.—This compound shows an antibacterial activity against the coliform organisms in the gastrointestinal tract of the dog intermediate between sulfasuxidine and sulfathalidine. Equivalent bacteriostasis occurs following the administration of 1.0 Gm. of sulfasuxidine per kilogram of body weight, 0.5 Gm. sulfa-4,5-dicarboxythiazole, or 0.25 Gm. Sulfathalidine.

Dogs tolerated repeated intravenous doses of 1.0 Gm. per kilogram of sulfa-4,5-dicarboxythiazole without evidence of acute or chronic toxic manifestations.

The intraperitoneal administration of the sodium salt of sulfa-4,5-dicarboxythiazole to mice gives the mortality figures shown in Table II. The lethal dose, 50, is between 1.0 and 1.30 Gm. per kilogram of body weight.

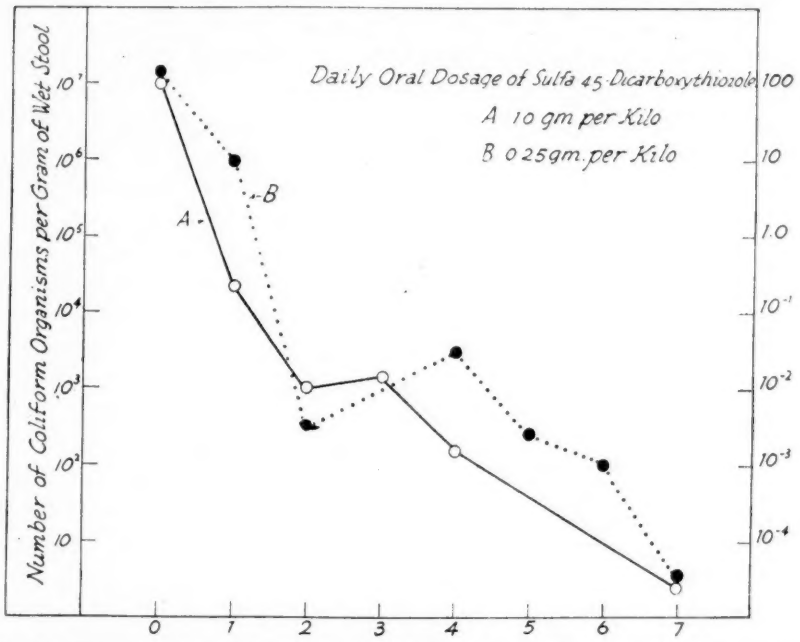

Daily Oral Dosage of Sulfa 4,5-Dicarboxythiozole
A 1.0 gm per Kilo
B 0.25gm. per Kilo
Number of Coliform Organisms per Gram of Wet Stool
A
B

Fig. 2.—Demonstrating that a daily oral dosage of 0.25 Gm. of sulfa-4,5-dicarboxythiazole per kilogram of body weight divided into six portions and given at four-hour intervals to the dog is equally as effective as 1.0 Gm. of the same drug similarily administered.

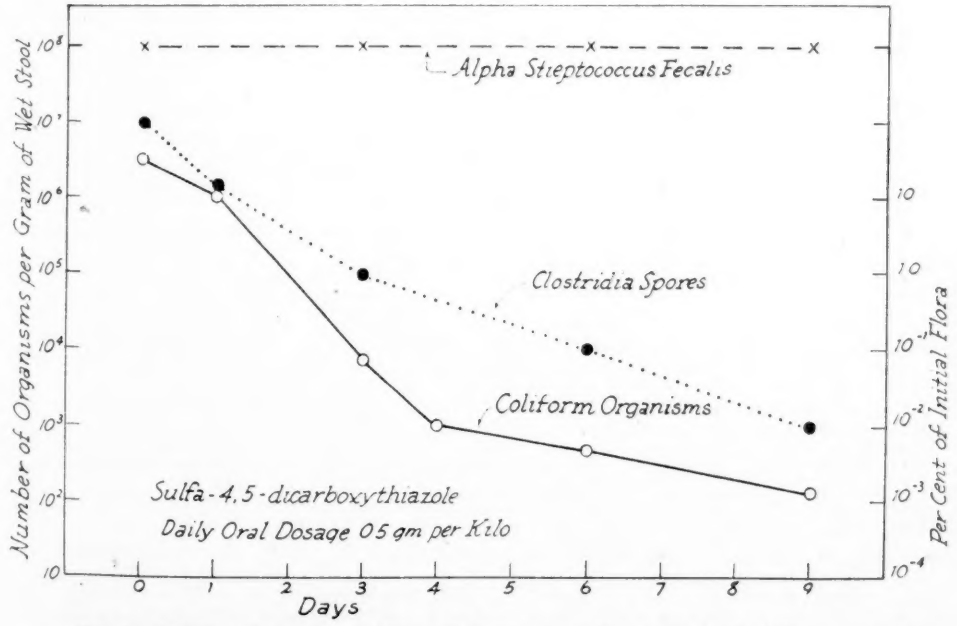


Fig. 3.—Showing the corresponding effect on anaerobic spores and coliform organisms upon the oral administration of sulfa-4,5-dicarboxythiazole to dogs in divided four-hour doses. This drug has no antibacterial action on the alpha *Streptococcus fecalis*.

TABLE II. THE INTRAPERITONEAL ADMINISTRATION OF THE SODIUM SALT OF SULFA-4,5-DICARBOXYTHIAZOLE TO WHITE MICE WEIGHING BETWEEN 29 AND 33 GRAMS

NUMBER OF MICE	DOSAGE (GM. PER KG.)	NUMBER OF MICE ALIVE AFTER FORTY-EIGHT HOURS	MORTALITY (PER CENT)
10	0.67	0	0
10	0.99	1	10
10	1.33	9	90
10	2.66	10	100

DISCUSSION

The carboxythiazoles contain free carboxyl groups resulting in compounds which are relatively strong acids and which readily form highly soluble salts. In this series of sulfonamides the primary para amino group is free in contradistinction to the acylation of this bacteriostatically active group in the acylated sulfonamides previously studied and represented by succinyl- and phthalylsulfathiazole.

While all of these carboxythiazoles have considerable antibacterial activity in the gastrointestinal tract, sulfa-5-carboxythiazole and sulfa-4,5-dicarboxythiazole possess the greater activity. All of these compounds maintain a moderately high concentration of the drugs in the blood. Comparison of the concentration of the drugs in the urine would indicate that sulfa-5-carboxythiazole is absorbed from the bowel more readily than is sulfa-4,5-dicarboxythiazole (see Table I).

Sulfa-4-methyl-5-carboxythiazole is the only member of this series that has been demonstrated to be relatively toxic. This toxicity is explainable on the basis that the compound readily splits out carbon dioxide to yield the corresponding 4-methylsulfathiazole which is toxic.

The nontoxic members of this series of sulfonamides, which significantly alter the bacterial flora of the gastrointestinal tract as indicated by the reduction of the coliform organisms, may prove to be useful therapeutic agents where it is desired to produce a general systemic effect as well as to alter the intestinal flora and maintain a relatively high concentration of the salt of a sulfonamide in the urinary tract.

SUMMARY

A series of carboxysulfathiazoles is studied and shown to possess relatively low toxicity and high antibacterial activity in the gastrointestinal tract of the dog. Sulfa-4-carboxythiazole, sulfa-5-carboxythiazole, sulfa-4,5-carboxythiazole, and sulfa-4-methyl-5-carboxymethylthiazole may possess desirable therapeutic properties.

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MEASUREMENT OF PULMONARY ARTERIAL PRESSURE IN DOGS

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STUDIES of the pressures developed in the pulmonary circulation have been rendered difficult because of the relative inaccessibility of the system. Direct measurements of pulmonary arterial pressures in experimental animals have been carried out,[1, 2] and in one report direct measurement of the pulmonary arterial tension was accomplished in man.[3] More recently, Johnson and coworkers[4] and Katz and Steinitz[5] have studied pulmonary arterial tensions (using special manometers) in trained dogs with closed thoraces. Their method appears to provide a reasonably accurate technique for recording such pressures, although requiring previous surgical preparation.

Our interest in alterations of pulmonary arterial tension in pulmonary embolism and pulmonary edema has led us to devise a method of registration of the tension which is simple and is performed without surgical manipulation of the thorax. The method is essentially a modification of that of Forssmann,[6] later utilized by others[7] for the recording of right intraventricular pressures.

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METHOD AND RESULTS

The technique entails the passage of a soft rubber *urethral* catheter into the right ventricle; the catheter then is carried into the pulmonary artery with the blood current.

Dogs weighing from 14 to 18 kilograms are anesthetized with veterinary nembutal. The *right* external jugular vein is isolated, and one common carotid artery is dissected free for subsequent registration of systemic arterial pressure. The animals are laid, left side down, on a wooden rack and are placed on the horizontal table of a fluoroscope. This position facilitates passage of the catheter through the pulmonary orifice. Each step of the procedure is successively examined through the fluoroscopic screen.

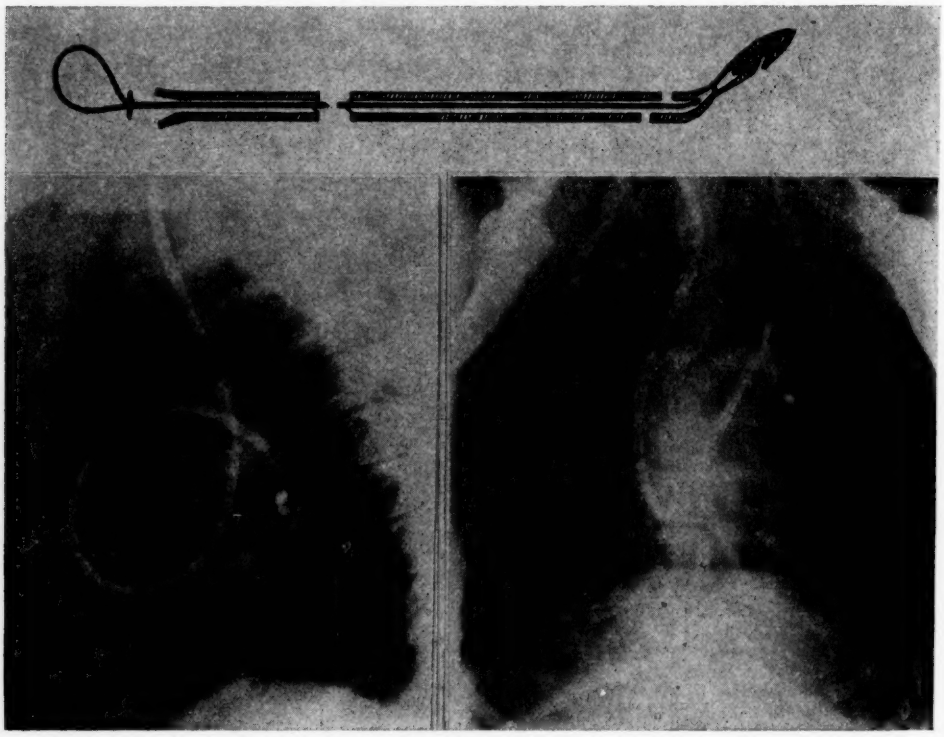

Fig. 1.—Top, Diagram of catheter in cross section. A bead of lead foil is inserted in the terminal aperture and the catheter is ligated. Proximal to the ligature two holes are shown in the wall of the tube. The beaded end of the guide wire is thrust against the constriction in the tube. Bottom, Roentgen cardiac shadows of dog with catheter tip in the pulmonary artery, lateral and anteroposterior views.

French No. 8 urethral catheters are generally suitable, although in larger dogs a No. 10 size may be used. The catheter is tightly ligated immediately proximal to the side aperture, and a bead of lead is placed in the aperture to enhance fluoroscopic identification of the catheter tip. Two or three small round holes are burned or cut into the catheter wall proximal to the ligature. A straight "guide wire" of spring steel, 42 cm. long, is provided with a small brass bead at its end; the wire is angulated for 5 mm. at its tip to facilitate passage through the tricuspid valve orifice (see Fig. 1). The guide wire is lubricated with mineral oil before insertion into the catheter; a thin coating of beeswax is applied to the exterior of the tube. The dogs are heparinized before the procedure is begun.

In most instances the catheter (with the guiding wire) is readily passed through an incision in the jugular vein into the superior vena cava and, with

little manipulation, into the right ventricle. The wire is withdrawn inside the catheter for from 2 to 3 cm., and the catheter is then introduced further with slight rotary motion over the wire into the ventricle. With few exceptions, the catheter tip is observed to follow the septal border of the ventricle and to pass directly into the pulmonary artery. If introduced far enough into a branch of the pulmonary artery, the end of the catheter apparently lies outside of, and behind the cardiac shadow.

Thus placed, the open end of the catheter is connected by pressure tubing to a Wiggers (optical) manometer. A glass cannula is tied into the common carotid artery, and the systemic blood pressure is recorded on a second Wiggers manometer. Suitable optical systems reflect light beams from the manometric mirrors into an electrocardiographic photokymograph, both curves being recorded on the same bromide film. Tests for adequacy and standardization of the manometric membranes are carried out in the usual way.[1]

Examples of pressure curves obtained from the pulmonary artery, together with systemic arterial pressures, are shown in Fig. 2. The catheter tip, lying in the main pulmonary arterial trunk, or near the orifice of either branch, is

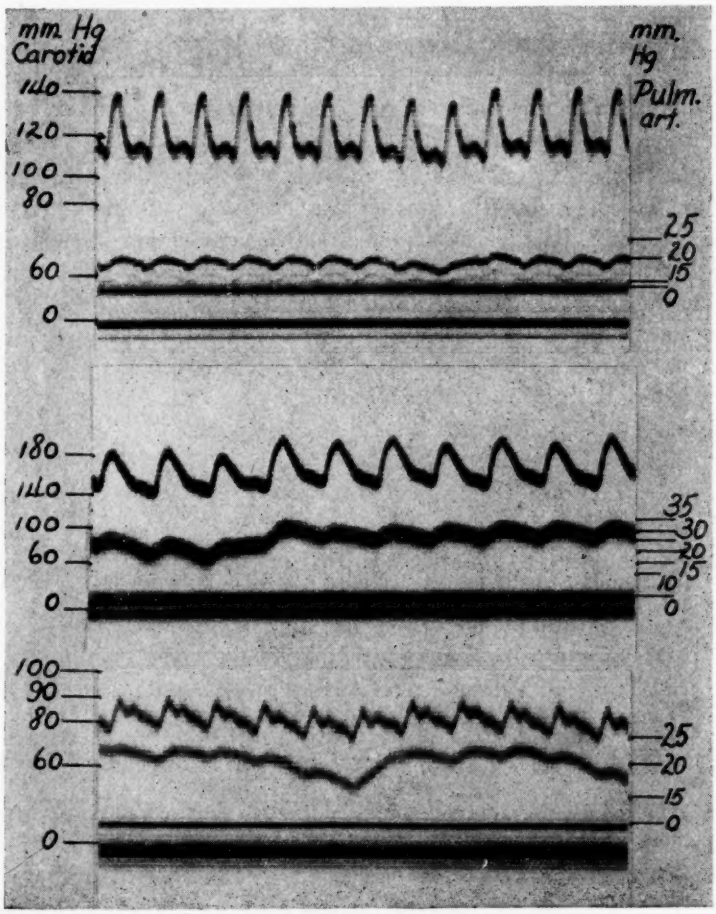

Fig. 2.—Examples of optical recording of pulmonary arterial and systemic arterial (carotid) pressures from three dogs. The upper curve is the recording of systemic arterial blood pressure; the lower curve, of the smooth contour, is that of pulmonary arterial tension. The standard light beam deviations are noted in the left and right margins, respectively. The periodic abrupt falls in pulmonary arterial pressure occur during inspiration. The films are recorded at a constant paper speed of 1 cm. in 0.4 sec.

in the axial stream. Accordingly, the longitudinal pressure curves obtained are smooth in contour and are devoid of abrupt and minute oscillations. The curves are in general conformity to those recorded by Katz and Steinitz[5] and others.[4]

DISCUSSION

A question may be raised as to whether the passage of the catheter through the pulmonary valve produces pulmonary insufficiency, at the sacrifice of diastolic pressure head. This possibility was readily investigated. In anesthetized dogs, with the thorax *opened,* an optical manometer was connected to a cannula tied into a small lateral branch of the pulmonary arterial system, and the pressure was recorded. Thereafter, the urethral catheter was introduced into the pulmonary artery by the technique described. The pulmonary arterial tension through the catheter was recorded on the same manometer. With either of these procedures, the mean arterial tensions were the same; the pulse pressure measured through the catheter (longitudinal pressure) was slightly lower than that recorded from a side branch because minute abrupt oscillations were not present. It appears probable that no leak occurs in the valve and that the resilient valve leaflets form an effective seal around the tube.

The manometric system, incorporating the catheter, seemed adequate for this purpose. The sudden application of pressure to the system (100 mm. Hg or more) caused as prompt and complete a deviation of the light beam as was recorded when the same pressure was applied to the manometer through ordinary pressure tubing. Lateral distention appeared to be nil in the catheters.

In numerous trials, verified by autopsy, it was found that, when the catheter tip lies immediately within the cardiac shadow in the pulmonary artery (right or left lateral view), it is in the main trunk of the artery or in the orifice of the left main branch. If the catheter is permitted to proceed farther into one of the lobar branches, it may become impacted in the lumen so that the pressure pulses are not obtained. In addition, it was found that the method is not satisfactory in very small animals, as the catheter will not loop sharply in a small right ventricle and the tip cannot be pushed to the pulmonary orifice; the tube coils upon itself within the heart. As a further precaution, overanesthetization of the animals is to be avoided because of the likelihood of the heart becoming hypodynamic or because respiration may become so altered as to distort the pulmonic pressure readings.

This method lacks the advantage of that of Johnson and co-workers[4] and of Katz and Steinitz[5] in that it requires anesthetization of the dog. In experimentation where anesthesia is desired, the method is advantageous in that it is simple to perform and all previous intrathoracic surgical manipulation is eliminated. It is suggested, further, that the technique might be adapted for the measurement of pulmonic arterial tension in man.

SUMMARY

A method is described for the insertion of a soft rubber urethral catheter into the right ventricle and thence into the pulmonary artery of dogs. Pulmonary arterial pressure may be recorded by an optical manometer. The simplicity of the procedure renders it advantageous where such experimentation on the animal is carried out under anesthesia.

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LIVER FUNCTION STUDIES ON SOLDIERS UNDER PROLONGED ATABRINE ADMINISTRATION

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ANIMAL studies have revealed that orally administered atabrine produces liver damage.[1, 2] To be sure, the doses employed were massive and in no way to be compared with the daily intake of our soldiers. However, since in this theater of operations, atabrine administration has been in effect for a considerable time the question arose as to whether small amounts of the drug taken over a long period of time might produce a subclinical hepatic damage which could be detected by sensitive liver function studies.

To this end, we took fifty ambulatory Negro soldiers who had been taking 0.1 Gm. of atabrine daily six days a week for about eighteen months. Only those soldiers who never had had clinical malaria, syphilis, scrub typhus, or jaundice were included in the study. The tests employed were icterus index, urine urobilinogen, fibrinogen, serum cholesterol partition, and the oral hippuric acid test. Total serum lipid determinations were performed where the total serum cholesterol values were unusually high. As a check on the fibrinogen results, this determination was also performed on fifty troops who had been taking atabrine only from one to three months.

Several months later, bromsulfalein, cephalin flocculation, and galactose tolerance tests were performed on fifty more soldiers (white and Negro). The latter likewise had been taking atabrine for from twenty to twenty-four months and had no previous history of liver damage. As a control of the sensitivity of the cephalin flocculation test, determinations were performed on twenty-five soldiers who had been taking atabrine for from one to three months. At a still later date, twenty-five additional bromsulfalein tests were performed on soldiers who had been taking atabrine for thirty months.

METHODS

Plasma fibrinogen was determined colorimetrically.[3-5] Icterus index was determined by the method of Meulengracht.[6] Urine urobilinogen was determined quantitatively by the method of Sparkman.[7] The oral hippuric acid test was

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performed according to the method of Quick,[8] as modified by Kraus and Dulkin.[9] Total serum cholesterol was measured colorimetrically,[10] while free cholesterol was determined essentially by the method of Schonheimer and Sperry.[11] The total lipid content of serum was determined gravimetrically.[12, 13] The bromsulfalein test was performed by injecting 2 mg. of bromsulfalein per kilogram of body weight[14] and by measuring the retention at the end of eighteen minutes.[15] For the cephalin flocculation test, the original method of Hanger[16] was used. The galactose tolerance test was performed by the method of Janney and Isaacson.[17]

RESULTS

Icterus index results were all normal, lying between 4 and 8 units. Urine urobilinogen was considerably less than the upper normal value of 8 mg. per cent; the results lay between 0.7 and 3.6 mg. per cent. Serum cholesterol partitions were also normal; the values obtained all were within the normal limits of from 21 to 29 per cent of free cholesterol. Of the fifty oral hippuric acid liver function tests performed, excretions of less than 3 Gm. were obtained in three subjects. Over one-half of the plasma fibrinogen valves were less than 0.3 Gm. per cent. However, of fifty control observations, 32 per cent were likewise less than 0.3 Gm. per cent. Therefore, the low plasma fibrinogen cannot be attributed to the effect of atabrine, but to some other unexplained cause. Galactose tolerance studies were all normal. In every instance, 3 Gm. or less of galactose were recovered in the urine within five hours. No retention of bromsulfalein was observed in any case, not even in those men who had been taking atabrine for thirty months.

At the end of twenty-four hours the cephalin flocculation tests gave a precipitation of 2 plus or greater in 46 per cent of the men, while of the twenty-five control determinations performed, a precipitation of 2 plus or greater was obtained in seven of the subjects. Since almost as high a percentage of positive results was obtained among the controls as among the soldiers studied, it must be concluded that in our hands the cephalin flocculation test was too sensitive and that the positive results obtained are not indicative of hepatic dysfunction. It might be added that the tubes were exposed to light in the laboratory after being set up. This might account for the increased sensitivity.

Consequently, of the hepatic function tests performed, only the hippuric acid test gave results indicative of hepatic dysfunction, and then only in a very few cases. This by itself cannot be considered significant. Therefore, it may be concluded that no subclinical liver damage could be detected among the soldiers that were studied.

Of the total cholesterol results obtained on the fifty Negro soldiers, more than one-third were higher than the usually accepted upper normal limit of 275 mg. per cent. These findings were not expected, and as a check, total serum lipid determinations were performed. In every instance where the cholesterol was high, a slightly elevated serum lipid was obtained. However, the total cholesterol values obtained on the second series of fifty soldiers ran much lower, only one being higher than 275 mg. The only explanation that can be offered for this difference is that the second series of determinations was performed on fasting blood specimens, while blood for the first series was drawn from two to three hours after breakfast.

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SERUM PROTEINS IN PORTAL CIRRHOSIS

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THE characteristic changes in the serum proteins in cirrhosis of the liver are a fall in albumin and a rise in globulin, leading frequently to a reversal of the albumin/globulin ratio. The parts played by the euglobulin and pseudoglobulin fractions in this change in serum globulin are described and their significance discussed.

MATERIAL

Cirrhosis of the liver is a common disease in Iraq. It is found principally, though not invariably, among the poor, and alcohol plays no part in its etiology. The course of the disease and the post-mortem findings resemble closely the Laennec's cirrhosis found in western countries. A detailed description of the condition has been given elsewhere.[1]

The thirty-six cases presented were male patients. The liver was small and ascites was present in all patients. Thus, in all, the disease was well developed. The difficulties in diagnosis in the early, pre-ascitic stage, particularly

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great in a country where other diseases affecting the liver are common, made unequivocal diagnosis desirable in a preliminary investigation. The Wassermann reaction was negative in twenty-eight and positive in eight of the patients.

Some difficulty was experienced in obtaining normal serum protein figures for comparison since the class concerned is commonly affected by gross malnutrition, chronic malaria, or conditions such as ankylostomiasis, leading to anemia. The results of estimations on normals are, therefore, presented in two groups, twelve apparently normal hospital patients and twelve medical students.

Total protein and *albumin* were estimated by the method of Greenberg,[2] and *euglobulin* by the following method derived from Proske and Watson.[3] Euglobulin was precipitated in a conical centrifuge tube by the addition of 0.2 c.c. serum to 6 c.c. 14 per cent sodium sulfate solution. After standing for twenty-four hours at 37° C., the tube was centrifuged, the precipitate washed twice with 3 c.c. portions of 14 per cent sodium sulfate, dissolved in 4.7 c.c. soda (1 c.c. 5 N. soda diluted to 23.5 c.c. with distilled water) and allowed to stand in boiling water for ten minutes. A standard was prepared in a 25 c.c. cylinder containing 2 c.c. standard tyrosine solution (0.20 Gm. tyrosine in 1,000 c.c. N/10 hydrochloric acid), 20.5 c.c. water and 1 c.c. 5 N. soda. The standard and the unknown were cooled to the same temperature in cold water and 0.3 c.c. Folin's phenol reagent added to the unknown and 1.5 c.c. to the standard. The volume of the unknown was made up to 5 c.c. and, after thorough mixing, compared in a colorimeter. In all the protein estimations, results were read from a curve constructed by reading a number of standard tyrosine solutions against a standard prepared as above.

The chromogenic power of euglobulin with Folin's phenol reagent was determined by sixteen parallel nitrogen estimations by micro-Kjeldahl. For calculating grams protein from grams N_2, the customary factor, 6.25, was used, although no reports on the nitrogen content of euglobulin could be found. The results are shown in Table I. The factor for converting grams tyrosine into grams euglobulin is therefore 10.15, and there is a maximum error of 6 per cent. The difference between this factor and the factor given by Greenberg for globulin, namely 14.4[4] is caused by the use of heat in dissolving the euglobulin precipitate in soda.

TABLE I. THE CHROMOGENIC POWER OF EUGLOBULIN WITH FOLIN'S PHENOL REAGENT

Column (a): Grams tyrosine colorimetrically equivalent to euglobulin present in 100 c.c. serum.
Column (b): Grams euglobulin per 100 c.c. serum.

CASE	COLORIMETRIC (A)	KJELDAHL (B)	B/A
1	0.142	1.36	9.6
2	0.094	0.96	10.2
3	0.147	1.53	10.4
4	0.052	0.56	10.8
5	0.108	1.09	10.1
6	0.089	0.88	9.9
7	0.072	0.76	10.5
8	0.084	0.88	10.5
9	0.096	0.95	9.9
10	0.098	0.94	9.6
11	0.067	0.71	10.6
12	0.080	0.86	10.7
13	0.150	1.55	10.3
14	0.068	0.69	10.1
15	0.052	0.52	10.0
16	0.139	1.37	9.9
Average			10.15

From the estimations described, the quantities of the protein fractions were obtained by multiplying by the following factors: albumin, 16.6; globulin, 14.4; euglobulin, 10.15. Total protein was obtained by addition and pseudoglobulin by subtraction.

RESULTS

The results are presented in Table II. Comparison of the two groups of normal subjects show that the better nutritional state of the students is reflected in the higher values of all protein fractions. The figures for the two groups of patients with cirrhosis are in close agreement. They can, therefore, be considered together since there is also no clinical reason for separating them.[1]

When the normal and cirrhotic subjects are compared, the fall in albumin, rise in globulin, and reversal of the A/G ratio is apparent. The euglobulin is

TABLE II. SERUM PROTEIN PARTITION IN NORMAL SUBJECTS AND IN PATIENTS WITH PORTAL CIRRHOSIS

NORMAL								CIRRHOSIS							
CASE	TOTAL PROTEIN (GM./100 C.C.)	ALBUMIN (GM./100 C.C.)	GLOBULIN (GM./100 C.C.)	EUGLOBULIN (GM./100 C.C.)	PSEUDOGLOBULIN (GM./100 C.C.)	ALBUMIN : GLOBULIN	EUGLOBULIN : ALBUMIN	CASE	TOTAL PROTEIN (GM./100 C.C.)	ALBUMIN (GM./100 C.C.)	GLOBULIN (GM./100 C.C.)	EUGLOBULIN (GM./100 C.C.)	PSEUDOGLOBULIN (GM./100 C.C.)	ALBUMIN : GLOBULIN	EUGLOBULIN : ALBUMIN
1	5.8	3.8	2.0	0.32	1.7	1.9	0.08	25	4.3	1.6	2.7	0.78	1.9	0.6	0.49
2	7.5	4.3	3.2	0.22	3.0	1.3	0.05	26	5.8	2.4	3.4	0.87	2.5	0.7	0.36
3	6.7	4.2	2.5	0.26	2.2	1.7	0.06	27	6.4	1.0	5.4	1.61	3.8	0.2	1.61
4	7.0	4.3	2.7	0.28	2.4	1.6	0.07	28	6.0	1.7	4.3	2.29	2.0	0.4	1.34
5	7.7	3.5	4.2	0.16	4.0	0.8	0.05	29	8.1	3.2	4.9	1.17	3.7	0.6	0.37
6	6.2	3.9	2.3	0.17	2.1	1.7	0.04	30	5.1	2.3	2.8	0.77	2.0	0.8	0.33
7	7.3	3.7	3.6	0.32	3.3	1.0	0.09	31	6.4	1.4	5.0	1.27	3.7	0.3	0.91
8	5.6	3.5	2.1	0.09	2.0	1.7	0.03	32	8.1	3.5	4.6	1.59	3.0	0.8	0.45
9	7.2	4.2	3.0	0.42	2.6	1.4	0.10	33	6.2	2.5	3.7	0.73	3.0	0.7	0.29
10	7.0	5.2	1.8	0.19	1.6	2.9	0.04	34	7.1	2.0	5.1	1.27	3.8	0.4	0.64
11	6.7	4.1	2.6	0.23	2.4	1.6	0.05	35	7.9	3.2	4.7	1.79	2.9	0.7	0.56
12	5.7	3.8	1.9	0.65	1.2	2.0	0.17	36	6.7	1.3	5.4	1.82	3.6	0.2	1.40
								37	5.5	1.9	3.6	1.21	2.4	0.5	0.64
Av. (1 to 12)	6.70	4.04	2.66	0.28	2.38	1.52	0.07	38	7.8	2.7	5.1	2.24	2.9	0.5	0.83
								39	7.7	3.2	4.5	1.64	2.9	0.7	0.51
13	7.4	4.8	2.6	0.26	2.3	1.8	0.05	40	7.1	2.7	4.4	1.41	3.0	0.6	0.52
14	8.1	5.0	3.1	0.53	2.6	1.6	0.10	41	6.5	1.9	4.6	1.73	2.9	0.4	0.91
15	6.9	3.6	3.3	0.20	3.1	1.1	0.06	42	7.1	2.5	4.6	1.81	2.8	0.5	0.72
16	8.4	5.5	2.9	0.18	2.7	1.9	0.03	43	6.5	1.6	4.9	1.98	2.9	0.3	1.24
17	8.2	5.1	3.1	0.55	2.5	1.6	0.10	44	5.4	1.2	4.2	1.73	2.5	0.3	1.44
18	8.3	5.0	3.3	0.36	2.9	1.5	0.07	45	6.6	2.3	4.3	1.26	3.0	0.5	0.55
19	6.7	4.3	2.4	0.21	2.2	1.8	0.05	46	8.3	2.7	5.6	2.02	3.6	0.5	0.75
20	6.9	4.5	2.4	0.37	2.0	1.9	0.08	47	6.7	2.2	4.5	1.01	3.5	0.5	0.46
21	7.6	4.6	3.0	0.39	2.6	1.5	0.08	48	7.5	1.9	5.6	1.54	4.1	0.3	0.81
22	6.0	3.8	2.2	0.49	1.7	1.7	0.13	49	6.1	2.2	3.9	0.75	3.1	0.6	0.34
23	8.2	5.5	2.7	0.43	2.3	2.0	0.08	50	7.7	2.4	5.3	1.22	4.1	0.5	0.51
24	8.2	5.1	3.1	0.59	2.5	1.6	0.12	51	6.2	2.0	4.2	1.07	3.1	0.5	0.54
								52	5.8	1.7	4.1	1.30	2.8	0.4	0.77
Av. (13 to 24)	7.57	4.73	2.84	0.38	2.46	1.67	0.08	53	7.1	2.0	5.1	1.97	3.1	0.4	0.99
								54	5.7	1.5	4.2	0.91	3.3	0.4	0.61
								55	6.4	2.8	3.6	1.02	2.6	0.8	0.36
								56	6.5	2.4	4.1	1.16	2.9	0.6	0.48
								57	6.9	2.6	4.3	1.74	2.6	0.6	0.67
								58	7.2	2.0	5.2	1.71	3.5	0.4	0.86
								59	7.8	3.5	4.3	0.92	3.4	0.8	0.26
								60	4.7	2.3	2.4	0.74	1.7	1.0	0.32
Av. (1 to 24)	7.14	4.39	2.75	0.33	2.42	1.60	0.08	Av. (25 to 60)	6.64	2.23	4.41	1.38	3.03	0.51	0.62

Cases 1 to 12, Normal patients.
Cases 13 to 24, Normal students.
Cases 25 to 52, Cirrhosis (Wassermann reaction negative).
Cases 53 to 60, Cirrhosis (Wassermann reaction positive).

considerably raised in the patients with cirrhosis, a point noticed by Gutman and Wise,[5] and in all subjects is outside the normal range; the average is four times the normal average. The range of pseudoglobulin values in the two groups is the same, although the averages show a small rise which is statistically significant. The calculation of the euglobulin/albumin ratio gives a figure which is, on the average, eight times as great as normal in cirrhosis.

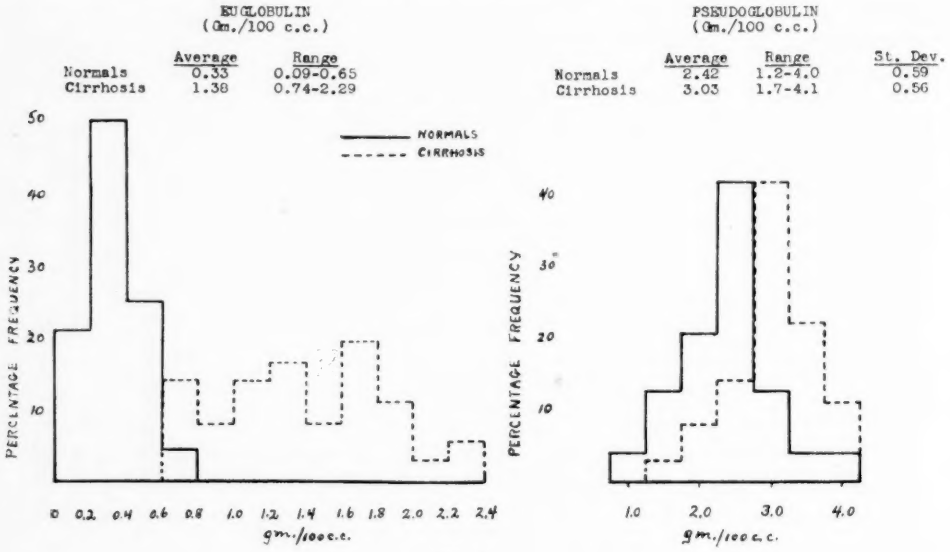

Fig. 1.

DISCUSSION

The diet of the section of the community in which cirrhosis of the liver is most common in this country is deficient in protein, and patients with cirrhosis are averse to taking food containing much protein. Since animals on a low protein diet develop low serum albumin values,[6] it might be thought that the low serum albumin observed was dietetic in origin. However, four patients with cirrhosis, kept for two weeks on a high protein diet, showed no rise in serum albumin (Table III). Further experiments would be needed to ascertain if failure to absorb digested protein plays any part in the production of these changes.

The separation of serum proteins by salting out raises the question of the existence of these components as separate entities in serum. A change in the serum protein fractions in disease must reflect either a difference in the proteins themselves or the presence of substances that affect their precipitation reactions.

TABLE III. EFFECT OF A HIGH PROTEIN DIET ON THE SERUM PROTEINS OF FOUR PATIENTS WITH PORTAL CIRRHOSIS

CASE	TOTAL PROTEIN	ALBUMIN	GLOBULIN	EUGLOBULIN	PSEUDO-GLOBULIN
1(a)	6.8	2.2	4.6	1.3	3.3
(b)	6.3	1.7	4.6	1.9	2.7
2(a)	5.8	1.7	4.1	1.3	2.8
(b)	5.7	1.8	3.9	1.1	2.8
3(a)	5.5	1.8	3.7	1.6	2.1
(b)	5.2	1.3	3.8	1.8	2.0
4(a)	4.6	1.2	3.4	1.0	2.4
(b)	4.4	1.0	3.4	1.0	2.4

(a) On admission.
(b) After two weeks on high protein diet.

Investigations by electrophoresis and the failure of attempts to change the A/G ratio by "environmental disturbances"[7] make the former more likely. The results presented here suggest that in cirrhosis a failure in the production of albumin is largely made good by increased euglobulin production. The serum euglobulin is known to be raised in a number of very different conditions, but no common factor has, as yet, been found. A search for other conditions in which these two fractions vary inversely might further elucidate the problem.

In the investigation of cases of cirrhosis of the liver, the estimation of serum euglobulin has advantages over the estimation of total globulin. Deviation from the normal is larger, and it might be expected to provide a more sensitive index of the abnormal protein metabolism of this disease. Moreover, the estimation is simple, and turbidometrically the measurement can be made very rapidly. However, a raised serum euglobulin should be used with caution as evidence of cirrhosis until more precise information is available as to the effect of liver diseases on euglobulin formation. Calculation of the euglobulin/albumin ratio gives an even greater deviation from normal but in the interpretation of this, other conditions leading to a lowering of the serum albumin must be excluded.

SUMMARY

1. Total protein, albumin, euglobulin, and pseudoglobulin were estimated in the serum of thirty-six patients with portal cirrhosis and the results compared with those of twenty-four normal individuals.

2. Serum euglobulin was above normal in all the patients with cirrhosis, the average value being four times the normal average.

3. Serum pseudoglobulin was within the normal range, but the average was raised; this rise was significant.

4. The serum euglobulin and the euglobulin/albumin ratio may provide more sensitive indices of disordered protein formation in this disease than the total globulin and the A/G ratio.

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ANOXIA IN MALARIA

An Experimental Study on Ducks

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IT HAS been suggested that anoxia is an important factor in the mechanism of death in acute malaria. This theory was based upon a study of *Plasmodium falciparum* infection in a child,[1] *Plasmodium knowlsi* infections in monkeys, (2) and *Plasmodium lophurae* infections in ducks.[3] Recent studies have shown that transfusions will prolong the course of malaria in ducks but that they do not prevent the ultimate death of the birds from this disease.[4] The temporary improvement of the host following transfusions apparently results from a decrease in the anemia. Hill[5] concluded from her study on pigeons infected with *Plasmodium relictum* that death resulted from the anemia. Hewitt,[6] however, has stated that "No definite correlation has thus far been noted between the degree of anemia and fatal infections, except in the case noted above."

The relation of anemia produced by the malarial infection has been correlated in this study with the inability of the host to survive a diminution of oxygen pressure at simulated altitudes. It has also been observed that death is related to the severity of the anemia and is not directly related to the total number of parasites present at any time during the disease.

METHODS AND MATERIALS

Plasmodium lophurae was used in this study to infect young white Pekin ducks and young chicks obtained from a commercial hatchery. Parasites used for the inoculum were obtained from highly parasitized donor birds. The blood was mixed with an equal volume of a 2.0 per cent sodium citrate solution in physiologic saline. The parasitemia was followed by counting the number of parasitized cells per 500 red cells. Smears were stained with a combination of Giemsa and Wright stains. Standard methods were used in counting the red blood cells. Young erythrocytes were differentiated from adult red cells by the dark staining of the cytoplasm and the round nucleus.

Simulated high altitude was obtained by evacuating a tank of a capacity of 4 cubic feet with a vacuum pump. The rate of ascent and the levels of altitude were regulated by a needle valve through which a constant flow of air was admitted. The tank was equipped with a sight glass through which the birds were carefully observed during the entire experiment. Light was furnished by a 60-watt bulb located inside the chamber. The temperature of the interior was controlled by partially immersing the tank in a water bath and by permitting water to flow over the upper half. The average temperature was 28° C. The rate of ascent was 1,600 feet per minute during the first twelve minutes and then 600 feet per minute until either the desired altitude or the lethal altitude was reached.

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EXPERIMENTAL

1. *A Comparison of the Lethal Simulated Altitudes for Normal and Malarial Infected Birds.*—In the first series of experiments a total of sixty-four normal chicks and thirty-seven chicks infected with malarial blood were placed in the decompression chamber from twenty-four to seventy-two hours following inoculation. They were decompressed at the rate given until all the malaria-infected chicks were dead. In every instance the malaria-infected birds succumbed both at a lower altitude and following a shorter interval of exposure than did the normal controls. Normal birds died from anoxic anoxia with convulsions of a clonic type. The first symptoms of anoxia were sleepiness and inability to hold up the head. As the simulated altitude increased, the chick's head fell slowly to the floor, whereupon it was jerked erect. These movements were repeated. As the anoxia increased, the head was thrown rhythmically from side to side. This was the first sign of convulsions. If the bird was disturbed at this time, either by one of the other chicks or by movement, the final convulsions occurred immediately, while if the bird was undisturbed, the final convulsion did not occur for a minute or two. All deaths in normal and malaria-infected birds were the result of respiratory failure.

In a second series of experiments a total of twenty-six malaria-infected ducks and fifty-seven normal controls were placed in the decompression chamber. The behavior of the ducks was similar to that of the chicks with the exception that the symptoms of anoxia, convulsions, and death occurred at a higher simulated altitude in the ducks than in the chicks.

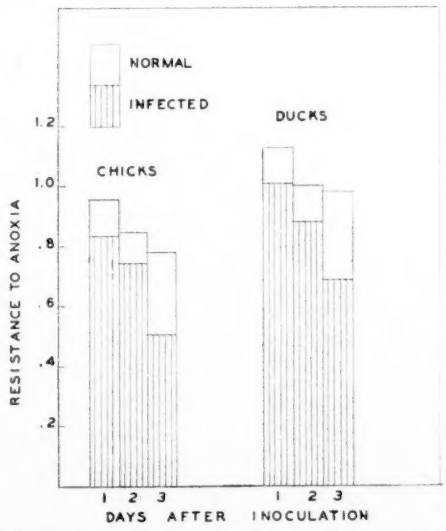

Fig. 1.—A comparison of the resistance to anoxia of malaria-infected and noninfected chicks and ducks. The resistance to anoxia, plotted on the ordinate, is calculated by multiplying the total altitude in feet times the total duration in minutes to which the bird was exposed to cause death.

The average results of these two series of experiments are shown in Fig. 1. The "resistance to anoxia," which is the product of the average simulated altitude at which convulsions and death occurred times the duration in minutes at this altitude, decreased with the daily progression of the infection. Since the experiments were terminated at the time of death of the last malaria-infected bird in the chamber, when approximately all the normal controls were

still alive, the level of resistance to high altitude of the normal birds only appears to fall on each successive day. Actually there was no appreciable decrease in resistance of the normals in this short interval. The parasitemia progressed rapidly from the third to the fifth day and death usually occurred by the sixth or seventh day of the disease.

2. *The Effect of Simulated Altitude on the Course of Malarial Infection in Birds.*—Normal ducks were decompressed to a simulated altitude of 20,000 feet and kept at this altitude for three or four days. They developed a polycythemia of 4 million red cells in comparison to the normal of 2.5 million. The peak of the polycythemia was reached in three days when the ducks were kept at a level of 20,000 feet.

In a third series of experiments, eight ducks were inoculated with malaria and the group was divided. One-half was placed in the decompression chamber and slowly decompressed to approximately 20,000 feet. The second half was kept at normal atmospheric pressure. Red cell counts, the number of young cells, the degree of parasitemia per 500 red cells, and the total number of parasitized cells per cubic millimeter were followed daily for five days in all of these birds. The average results obtained in this study are shown in Fig. 2.

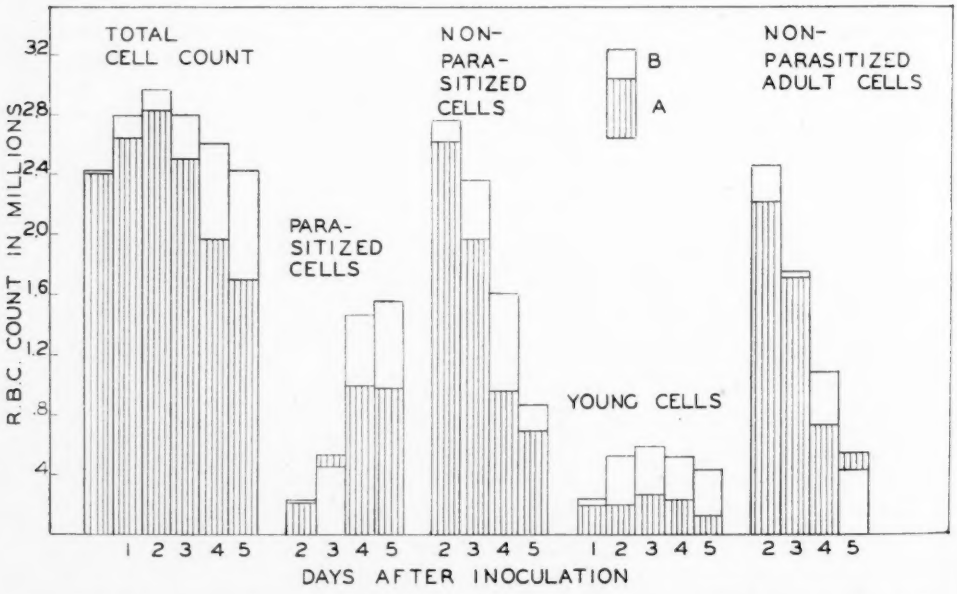

Fig. 2.—A comparison of total red blood cell counts, total parasitized cell counts, total nonparasitized cell counts, total young cell counts and the total nonparasitized adult cells of, *A*, a group of malaria-infected ducks kept at atmospheric pressure and, *B*, a group of malaria-infected ducks kept at simulated altitude of 20,000 feet for five days.

The total red cell count in Fig. 2 shows an increase on the second day and a subsequent fall thereafter in the birds kept in the chamber and also in those kept at normal atmospheric pressure. The rate of drop is considerably greater, however, in the latter group. The total number of parasitized cells is one-third greater in those birds kept at high altitudes when compared with those kept at normal atmospheric pressure. The total number of unparasitized cells decreases sharply in both groups as the disease progresses. The complete loss of these cells seems evident from the rate at which they disappear, as shown in Fig. 2. The formation of young cells is more rapid in the birds kept at high altitude. It will be noted from these data that the increase in number of the new cells in the

birds at simulated altitudes has not prevented the rapid disappearance of the old cells, and furthermore, these young cells cause the apparent slower rate of fall in the total number of remaining normal cells. It will be noted also that the rate of disappearance of adult erythrocytes is no greater in the birds at simulated altitude than it is in those birds kept at normal atmospheric pressure even though the total parasite count is higher in the former group.

Six ducks in another experiment were infected with malaria and four were taken to a simulated altitude of 20,000 feet. On the fifth day two of these birds were removed from the chamber. The total red cell count, the degree of parasitemia, and the total number of parasitized cells per cubic millimeter were followed in each of these ducks during the entire course of the disease.

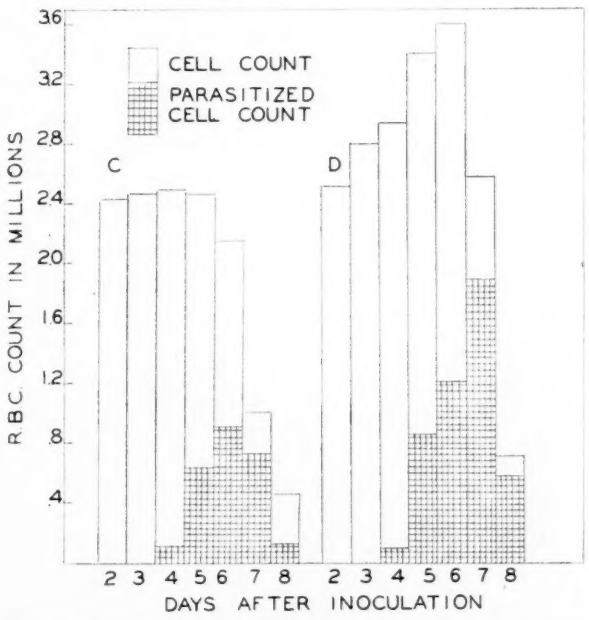

Fig. 3.—A comparison of the total red blood cell counts and total parasitized cell counts in, *C*, a malaria-infected duck kept at atmospheric pressure until death and, *D*, a malaria-infected duck kept at an altitude of 20,000 feet until death.

In Fig. 3 are shown the total number of red cells and the total number of parasitized cells in one of these ducks which died in the chamber at a simulated altitude of 20,000 feet and corresponding data on a control duck with malaria kept at atmospheric pressure which died on the same day. The malaria-infected bird kept at normal atmospheric pressure has less than half the total number of parasitized cells at the peak of the infection as compared with the bird kept at high altitude. Furthermore, one-third more red cells was destroyed in the bird kept at high altitude in the same length of time. The total number of parasitized red cells is approximately twice as great at the peak of the infection in the bird in the chamber as it is in the bird at atmospheric pressure. The total number of red cells in both birds falls to an extremely low level at the time of death. It may be as low as 200,000 or 300,000, or about one-tenth the normal count.

There were two ducks that survived the infection: one was kept at normal atmospheric pressure and the other was removed from the chamber on the fifth day after the inoculation. The data on these two birds are shown for comparison in Fig. 4. The number of parasitized red cells was somewhat higher in the bird

removed from the chamber. The level of the parasitized cell count at the depth of the anemia was approximately the same in the two birds and the rate of recovery was the same. The number of red cells destroyed between the peak of the infection and the time at which the smallest number of red cells was present is considerably greater in the bird which had been in the chamber than it is in the bird kept at atmospheric pressure.

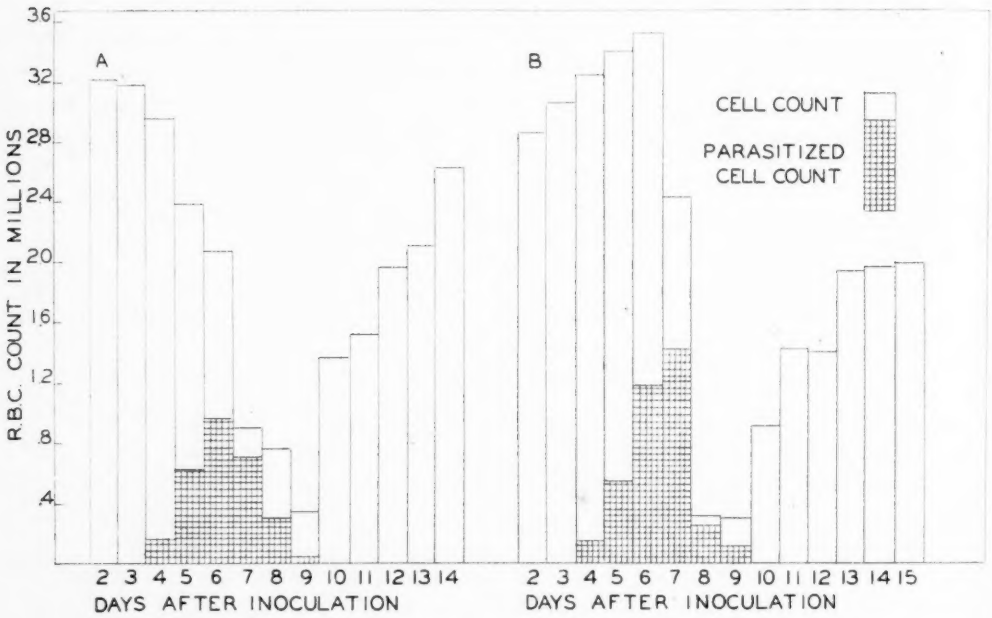

Fig. 4.—A comparison of the total red cell counts and the total parasitized cell counts of, *A*, a duck kept at normal atmospheric pressure and, *B*, a duck kept at an altitude of 20,000 feet for the first five days after the inoculation.

DISCUSSION

The results obtained in the first series of experiments indicate that birds with a mild malarial infection are definitely unable to withstand severe anoxia such as occurs at simulated high altitude. These birds were not obviously sick from the infection on the first, second, or third day after inoculation. The parasitemia was low on the third day in comparison with that at the peak of the infection. It is obvious, therefore, that ducks may show the effect of anemic anoxia before they reach the terminal stages of the infection. The sequence of the development of the symptoms of anoxia and the first indications of the convulsive state in the birds infected with malaria are identical to those occurring in birds not infected with malaria, except that the onset occurs in the former group at a lower altitude and after a shorter period of exposure. Ducks and chicks show the same sequence of development of symptoms to anoxia except that the first appearance of symptoms in the ducks occurs at higher altitude. It appears that the decrease in resistance to anoxia as observed in malaria-infected birds is the result of the inability of the oxygen transporting system to meet the increased demand accompanying the decrease in oxygen partial pressure.

The response of the erythrocyte-forming system in malaria-infected birds kept at normal atmospheric pressure is less than it is in infected birds kept at simulated altitudes. As a result of this earlier stimulation of the erythropoietic tissues the red blood cell count in the latter birds remains higher for a longer

period and the number of parasitized cells likewise increases. This increase in the number of parasitized cells may be explained by the fact that the parasites have a greater opportunity to parasitize red cells in the polycythemic birds. If the number of parasites decreases following the peak of the infection only as a result of phagocytosis by macrophages as recently stated by Taliaferro,[7] it would appear from our study that the phagocytosis of the parasitized cells is greatly diminished in the birds kept at high altitudes compared with birds kept at normal atmospheric pressure. The observation made by one of us that the course of the parasitemia in ducks infected with *P. lophurae* may be changed by transfusion with normal duck blood would suggest that factors other than immunologic ones play a significant role in the mechanism of the diminution in the number of parasites in this disease.[4] It also has been shown by McDougall[8] that the parasitemia in birds may be modified by inducing a state of hypoglycemia.

It would seem to us that the birds that survive a severe malarial infection may do so because the anemia develops slowly enough to cause a change in the environment that is unfavorable for the multiplication of the parasites before the anoxia resulting from the anemia becomes lethal for the host. If the decrease in the number of parasitized red cells were parallel to the decrease in the total red cell count, no bird would survive the infection. The fall in the number of parasitized cells is sharper than the fall in the number of red cells, as shown in Fig. 2.

The anemia in the chicks and ducks used in this study is extremely severe during the terminal stages of the disease. The great increase in the total number of parasitized cells in the birds kept at simulated altitudes did not produce an earlier death of the host. The failure of death to occur earlier in these birds would suggest that death in malaria is not dependent upon the total number of parasites present nor is it the result of a toxin produced by these parasites. It has been suggested that the plugging of capillaries by parasitized cells is an important factor in the mechanism of death in malaria.[9] It might be supposed that in the presence of an excessive number of both red cells and parasitized cells in the birds kept within the decompression chamber that capillary obstruction would occur earlier in the course of the disease. The plugging of capillaries by emboli, however, has not been observed in histologic studies made by one of us (R. H. R.) in cases of either malarial infection in man or experimental animals.

The data reported in this paper appear to lend support to the opinion that anoxia plays an important role in the mechanism of death in acute malarial infections.[1-3] The ducks in this study developed an anemia of such severity as to produce anemic anoxia preceding death. Furthermore, anoxia may be present in the malaria-infected ducks before the anemia becomes marked. This is indicated by the early death of the malaria-infected bird when placed at simulated high altitudes.

In considering the role of anoxia in acute malarial infections in man it should be remembered that the infection is accompanied by elevated temperatures and that the increment of oxygen utilization follows the normal Q_{10} for biologic reactions. The strain on the oxygen transporting system may be increased by the elevated temperature and contribute indirectly to the anoxia produced by the acute anemia.

SUMMARY

1. The resistance of ducks and chicks infected with malaria to anoxic anoxia is less than that of normal birds.

2. The increase in susceptibility to anoxia increases as the malarial infection progresses.

3. Ducks with a polycythemia develop an extremely high total parasitized red cell count which may be double that occurring in malaria-infected birds kept at normal atmospheric pressure.

4. All birds develop a severe anemia preceding death. The total red cell count may reach a level of from 250,000 to 300,000.

5. It is suggested that the few birds in this study that survive the inoculum, fatal for the majority of ducks, may do so because the rate of decrease of the number of parasitized cells does not parallel the fall in the total red cell count during the terminal phase of the infection.

6. The experimental observations made in this study support the opinion that anoxia is a significant process in the mechanism of death in birds infected with *P. lophurae*.

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ATTEMPTED TRANSMISSION OF HUMAN LEUCEMIA IN MAN

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THE problem of leucemia has been widely discussed in Forkner's monograph, *The Leukaemias and Allied Disorders,*[1] a book in which is summarized our knowledge of human and animal leucemia up to the year 1937. The advanced state of research into leucemia in mice and fowls, and the postulation of their principal identity with human leucemia because of clinical, symptomatologic, and morphologic analogies, makes it desirable to investigate the main features of leucemia in human beings. One important problem is that of the transmissibility of human leucemia. In mice and guinea pigs leucemia can readily

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be transmitted with cellular material in inbred strains, and it was in this way that the first difficulties of transmission were overcome. According to this experiment one would expect to have the best prospects of a take in man in transmission experiments in leucemic families or children of leucemic parents with leucemic material taken from a spontaneous case of leucemia of one of the members of the family or parents. Until now no family or children with leucemia have volunteered for such an observation and the studies to be described were conducted strictly on patients with a life prospect of two years or less. In mice, leucemia can be transmitted by intravenous, subcutaneous, or intramuscular injection of blood or other cellular leucemia material. In fowls leucemia can even be transmitted with cell-free material. In human beings there are only a few reports of intravenous injection of leucemia blood into other men.

Forkner mentions Schupfer[2] who injected, without result, the blood of a patient with chronic myeloid leucemia into three patients with carcinoma and also the blood of a man with acute leucemia into another patient with carcinoma. Moewes[3] attempted transmission with blood serum from a patient with leucemia into one with erythremia. Minot and Isaacs[4] transfused a patient with generalized lymphosarcomatosis with leucemic blood without result. Gamble[5] tried, without effect, to transmit lymphatic leucemia to a patient with myeloid leucemia by injecting 5 c.c. of blood intravenously. Gramen[6] accidentally used a donor for blood transfusion who a few days later developed acute leucemia; the recipient did not develop leucemia. R. Gosio[7] transfused into a patient with chronic lymphatic leucemia large quantities of blood from a patient with chronic myeloid leucemia with a high leucocyte count of 200,000 and 300,000, respectively. He studied the effect of the transfusion on the lymph nodules of the patient, taking biopsies at intervals. He reported proliferation of the histiocytes, increased reticulum fiber formation, and finally a tendency to scarring with repression of the leucemic infiltrations.

All these observations gave negative evidence without finally disproving the possibility of transmission. It was decided, therefore, to carry out similar investigations using a larger number of individuals.

ATTEMPTED TRANSMISSION TO PATIENTS WITH CARCINOMA AND OTHER CHRONIC DISEASES WITH A LIFE PROSPECT OF LESS THAN TWO YEARS

Technique.—Cellular suspensions of a lymph nodule or blood from the living patient or of suspension of splenic material (spleen removed post mortem) were injected subcutaneously. Ten grams of tissue from lymph nodule or spleen were ground with sterile sand and diluted with 30 c.c. of physiologic saline solution. The resulting suspension was spun for a few minutes at low speed and 2 c.c. of the supernatant emulsion containing numerous intact cells were injected subcutaneously into the forearm. The material used for injection was always fresh and was injected between twenty minutes and one hour after it had been taken from the patient. Post-mortem examinations were conducted immediately after death of leucemic patients and in cases of unavoidable delay in subsequent treatment, the spleen was kept at 4° C. after it was removed from the body. All material inoculated was bacteriologically sterile. The bloods of the recipients were examined before the inoculation of the leucemic material, daily afterward for a week, then periodically weekly, and after three months of observation, periodically monthly. Whenever possible a post-mortem examination was conducted on all patients inoculated.

So far, all results have been negative, possibly because most of our patients already had carcinoma, slight infection, etc. These negative results do not mean that leucemia is not transmissible; they show only that our trials were unsuccessful and that the technique and conditions should be altered.

The following are the protocols:

PROTOCOL 1.—Woman, 46 years of age. Acute myeloblastic leucemia. White blood cells on day of death, 800,000 cubic millimeter; hemoglobin, 2.9 Gm. per cent.

Fresh material from the spleen was injected into five patients within twenty minutes of the death of the leucemic patient, each receiving 2 c.c. subcutaneously in the forearm.

AGE (YR.)	SEX	SITE OF NEOPLASM	DEATH AFTER INOCULATION (DAYS)
59	M	Carcinoma of penis	26
58	M	Sarcoma of shoulder	13
43	F	Carcinoma of breast	39
45	F	Carcinoma of breast	88
82	F	Sarcoma of pelvis	31

Thick cellular suspension from the spleen kept for one hour at 4° C., 2 c.c., were injected subcutaneously into the forearm.

AGE (YR.)	SEX	NATURE OF DISEASE	DEATH AFTER INOCULATION (MO.)
76	F	Diabetes mellitus	28
73	F	Diabetes mellitus	24
38	F	Pernicious anemia	12
16	F	Aplastic anemia	8
53	F	Pernicious anemia	14

The spleen was kept at 4° C. overnight and was freshly ground up before injection; 2 c.c. of the cellular emulsion were injected subcutaneously into the left forearm.

AGE (YR.)	SEX	SITE OF LESIONS	DEATH AFTER INOCULATION	RESULT P.M.
33	F	Carcinoma of cervix	33 days	Negative
58	F	Carcinoma of bowel	30 days	Negative
45	F	Carcinoma of cervix	6 mo.	Negative
80	F	Carcinoma of bowel	26 mo.	Negative
41	F	Carcinoma of cervix	6 mo.	Negative
67	F	Carcinoma of cervix	5 mo.	Negative
77	F	Carcinoma of uterus	2 mo.	Negative
61	F	Carcinoma of breast	2 mo.	Negative
45	F	Carcinoma of cervix	6 mo.	Negative
68	F	Carcinoma of stomach	20 days	Negative
62	F	Carcinoma of pancreas	2 mo.	Negative
68	F	Carcinoma of breast	Still alive after 28 mo.	Negative
54	M	Carcinoma of skin	2 mo.	Results of blood and spleen examinations entirely negative; postmortem examination could not be performed
67	M	Carcinoma of stomach	6 mo.	
52	M	Carcinoma of stomach	1 mo.	
79	M	Carcinoma of skin	Still alive after 28 mo.	
61	M	Syphilis	Still alive after 28 mo.	
62	M	Carcinoma of rectum	7 mo.	
37	M	Carcinoma of bladder	4 mo.	

In all these cases clinically there was no increase in the leucocytes; regional lymph node and splenic enlargement did not occur.

PROTOCOL 2.—Man, 55 years of age. Subacute myeloid leucemia. White blood cells on day of death, 650,000 per cubic millimeter; hemoglobin, 4.2 Gm. per cent. Spleen removed five minutes after death; cellular emulsion inoculated twenty minutes after death. Two cubic centimeters subcutaneously injected into the forearm.

AGE (YR.)	SEX	NATURE OF DISEASE	DEATH AFTER INOCULATION	RESULTS
47	M	Heart failure	7 mo.	Entirely negative
49	M	Heart failure	13 days	
51	M	Aneurysm of aorta	20 days	
68	F	Carcinoma of skin	Still alive after 28 mo.	
70	F	Carcinoma of skin	Still alive after 28 mo.	
66	M	Carcinoma of skin	Still alive after 28 mo.	
74	M	Carcinoma of tonsil	30 days	
88	M	Carcinoma of nose	23 mo.	

PROTOCOL 3.—Boy, 12 years of age. Acute myeloid leucemia. White blood cells on day of death, 1,200,000 per cubic millimeter; hemoglobin, 3.0 Gm. per cent. Spleen removed ten minutes after death; inoculation twenty-three minutes after death.

AGE (YR.)	SEX	NATURE OF DISEASE	RESULTS
67	F	Carcinoma of skin	At post-mortem examination twenty-six months after inoculation no sign of leucemia
84	F	Carcinoma of skin	

PROTOCOL 4.—Woman, 52 years of age. Chronic myeloid leucemia with acute exacerbation. White blood cells, on day of death, 720,000 per cubic millimeter; hemoglobin 3.8 Gm. per cent. Spleen removed two minutes after death; inoculation eighteen minutes after death.

AGE (YR.)	SEX	NATURE OF DISEASE	RESULTS
60	M	Coronary occlusion	At post-mortem examination 14 months after inoculation no signs of a leucemia

In the following cases material from leucemic patients was injected intravenously.

PROTOCOL 5.—Blood from a patient with acute lymphatic leucemia, man, 37 years of age, blood Group A (II), with general enlargement of all lymph glands, tumor of the mediastinum and spleen, was injected intravenously into two patients with carcinoma of the esophagus. White blood cells on day of donation, 87,000 per cubic millimeter; hemoglobin, 6.7 Gm. per cent.

(1) Man, 55 years of age. Carcinoma of esophagus. Blood Group A (II).

(a) Injection of 50 c.c. of leucemic blood (white blood cells, 87,000).

(b) Injection eight days later of 80 c.c. of leucemic blood (white blood cells, 82,500). Death of patient occurred fourteen days after the first injection; at post-mortem examination no abnormality was detected. The daily blood examination until death and also the gross and microscopic necropsy examinations of the tissue were entirely negative.

(2) Man, 62 years of age. Carcinoma of esophagus. Blood Group A (II). Injection of 50 c.c. of leucemic blood (white blood cells, 82,000). Death of patient three days later. Daily blood examinations and post-mortem findings were negative.

PROTOCOL 6.—Woman, 36 years of age. Chronic myeloid leucemia. Blood Group B (III). White blood cells at time of injection, 279,000 per cubic millimeter; hemoglobin, 7.8 Gm. per cent. Intravenous injection of 50 c.c. of leucemic blood into a man, 60 years of age, Group B (III), suffering from coronary thrombosis with partial occlusion. Weekly blood examination did not show any abnormality for seven months. At post-mortem examination there was no evidence of leucemia.

PROTOCOL 7.—Man, 51 years of age. Chronic myeloid leucemia. Blood on the day of removal of lymph nodule showed white blood cells, 48,000 per cubic millimeter; hemoglobin, 3.43 Gm. per cent. An enlarged lymph nodule the size of an almond was removed with sterile precautions under local anesthesia. The nodule was gound up with sterile sand and saline. The mixture was spun at low speed and the supernatant cellular emulsion used for injections.

Two patients received 2 c.c. of the cellular emulsions subcutaneously in the forearm.

(1) Woman, 61 years of age. Carcinoma of the face.

(2) Woman, 82 years of age. Carcinoma of face.

There was no evidence of leucemia after thirty-six and thirty-four months of observation.

TABLE I

PROTOCOL	SEX	AGE (YR.)	TYPE OF LEUCEMIA OF DONOR	DURATION OF LEUCEMIA IN PATIENT		INOCULATION		BLOOD OF DONOR AT TIME OF INOCULATION								
				BEFORE INOCULATION	AFTER INOCULATION	MATERIAL USED	AMT. (C.C.)	Hb. (GM. %)	W.B.C. 10^3	POLYMORPHONUCLEARS	LYMPHOCYTES	EOSINOPHILES	BASOPHILES	MYELOCYTES	MYELOBLASTS	NUCLEATED REDS
1	F	46	Acute myeloblastic	6 days	—	Suspension of splenic material	2	2.9	800	1	–	–	–	2	97	–
2	M	55	Acute myeloblastic	122 days	—	Suspension of splenic material	2	4.2	650	8	–	–	2	10	77	3
3	M	12	Acute myeloblastic	2 days	—	Suspension of splenic material	2	3.0	1200	4	–	–	–	1	95	–
4	F	52	Chronic myeloid	13/12 mo.	—	Suspension of splenic material	2	3.8	720	16	–	4	1	3	72	4
5	M	37	Acute lymphatic	25 days	8 days	Blood	80 50	6.7	87	1	99	–	–	–	–	–
6	F	36	Chronic myeloid	14/12 mo.	20½/12 mo.	Blood	50	7.8	279	37	–	2	3	56	2	–
7	M	51	Chronic myeloid	21/12 mo.	½/12 mo.	Suspension of lymph nodule	2	3.43	48	2	–	4	6	12	70	6

ATTEMPTED TRANSMISSION TO OTHER PATIENTS WITH LEUCEMIA

An attempt was made to transmit myeloid leucemia to patients with lymphatic leucemia and vice versa in order to see whether patients with one type of leucemia are susceptible to the other type.

Technique.—The blood of patients with chronic leucemia was used for transmission at a time when the white blood count was high, liver, spleen, and glands were generally enlarged, and just before treatment was necessary; the recipients were generally in a relatively good state of health, their chronic leucemia being reduced to a considerable degree by previous irradiation and arsenic therapy. This arrangement was adopted in the hope of enabling the respective transmitted cells to gain a footing in the body. Blood was transmitted only within the same blood group or with compatible blood groups. Both patients were brought together, and 6 c.c. of blood was taken extravenously from the florid leucemia with 0.6 c.c. of 2 per cent citrated saline; it was immediately injected intravenously into the recipient (within a minute after it was drawn). The full blood count of donor and recipient was done at the time of transmission, and both were examined weekly during the following months. All patients involved in the study were under continuous supervision for more than twelve months and had had previous full blood counts done at weekly intervals.

Experiment.—

1. Man, 36 years of age. Chronic myeloid leucemia for thirty-five months; white blood cells, 122,000 per cubic millimeter; hemoglobin, 7.9 Gm. per cent; polymorphonuclears, 27 per cent; myelocytes, 33 per cent; myeloblasts, 21 per cent; eosinophiles, 2 per cent; and basophiles, 16 per cent. This patient was used as a donor for a man, 45 years old, who had chronic lymphatic leucemia of thirteen months' duration; hemoglobin, 9.2 Gm. per cent; white blood cells,

11,000 per cubic millimeter; polymorphonuclears, 10 per cent; lymphoblasts, 90 per cent. The patient had just completed a course of deep x-ray therapy over spleen and thorax.

2. Woman, 36 years of age. Chronic myeloid leucemia for sixteen months. White blood cells, 95,000 per cubic millimeter; polymorphonuclears, 30 per cent; myelocytes, 41 per cent; myeloblasts, 11 per cent; eosinophiles, 1 per cent; basophiles, 14 per cent; nucleated reds, 3 per cent. This woman served as donor for a man, 53 years of age, with chronic lymphatic leucemia of thirteen months' duration. Hemoglobin, 10.64 Gm. per cent; white blood cells, 5,300 per cubic millimeter; polymorphonuclears, 54 per cent; lymphoblasts, 46 per cent. The patient had just completed a course of deep x-ray therapy two days previously.

3. Man, 63 years of age. Chronic lymphatic leucemia for twenty-six months. White blood cells, 90,500 per cubic millimeter; hemoglobin 10.82 Gm. per cent; polymorphonuclears, 7 per cent; lymphoblasts, 93 per cent. He served as a donor for two patients:

(a) Woman, 36 years of age. Chronic myeloid leucemia for 19 months. Hemoglobin 8.26 Gm. per cent; white blood cells, 51,500 per cubic millimeter; polymorphonuclears, 54 per cent; myelocytes, 31 per cent; myeloblasts, 7 per cent; eosinophiles, 1 per cent; and basophiles 7 per cent. The patient had just completed the last treatment of a deep x-ray course of therapy.

(b) Man, 42 years of age. Chronic myeloid leucemia for forty-eight months. Hemoglobin, 6.8 Gm. per cent; white blood cells, 68,000 per cubic millimeter; polymorphonuclears 62 per cent; myelocytes, 30 per cent; myeloblasts, 3 per cent; basophiles, 5 per cent. The patient had completed the last treatment of a deep x-ray course of therapy six days previously. He had slight palpitation and sweating three minutes after the transmission but walked home unaided afterward.

4. Man, 63 years of age. Chronic lymphatic leucemia for twenty-six months. Hemoglobin, 10.92 Gm. per cent; white blood cells, 95,000 per cubic millimeter; polymorphonuclears, 7 per cent; lymphoblasts, 93 per cent. This man served as a donor for a young woman, 32 years of age, who had had chronic myeloid leucemia for nineteen months. Hemoglobin, 9.5 Gm. per cent; white blood cells 28,000 per cubic millimeter; polymorphonuclears, 45 per cent; myelocytes, 41 per cent; myelobasts, 1 per cent; eosinophiles, 6 per cent; and basophiles, 7 per cent. The patient had completed the latest course of irradiation therapy on the same day.

5. Man, 53 years of age. Chronic lymphatic leucemia for eleven months. Hemoglobin, 10.68 Gm. per cent; white blood cells 15,000 per cubic millimeter; polymorphonuclears, 15 per cent; lymphoblasts, 85 per cent. He served as a donor for a man, 36 years old, with chronic myeloid leucemia of thirty-six months' duration. Hemoglobin, 13.1 Gm. per cent; white blood cells, 13,500 per cubic millimeter; polymorphonuclears, 75 per cent; myelocytes, 5 per cent; myeloblasts, 1 per cent; basophiles, 16 per cent; lymphocytes, 2 per cent; and eosinophiles 2 per cent.

Results of Investigation.—Six months after the attempted transmissions, there was no evidence of success. Lymphatic leucemia did not develop in patients with myeloid leucemia, nor did myeloid leucemia develop in patients with lymphatic leucemia. Between forty and fifty days after the transmissions, all but one patient showed a flare-up of his respective type of leucemia without any apparent reason and without showing increased number of cells in the blood

characteristic of the transmitted type. This was remarkable in view of the fact that three of the patients had previously had rather regular intervals between the relapses of their chronic leucemia. The patients used to come in at three-, five-, and six-month intervals, respectively, for treatment, and now all came between the fortieth and fiftieth day after the experimental transmission. One would go too far in saying that the flare-up after fifty days was due to the transmission, because unexpected things happen always in leucemias, and common colds or other factors might have been responsible. Normal blood given in transfusion usually has the opposite effect and is frequently of beneficial effect in leucemia.

TABLE II

PROTOCOL	SEX	AGE (YR.)	DONOR'S LEUCEMIA	DONOR FOR RECIPIENT	AMOUNT OF BLOOD USED FOR TRANSMISSION (C.C.)	DONOR'S BLOOD AT TIME OF TRANSMISSION									DURATION OF DONOR'S LEUCEMIA IN RECIPIENT (MO.)		
						HEMOGLOBIN (GM. %)	W.B.C. 10^3	POLYMORPHONUCLEARS	LYMPHOCYTES	EOSINOPHILES	BASOPHILES	MYELOCYTES	MYELOBLASTS	NUCLEATED REDS	BEFORE INOCULATION	AFTER INOCULATION	TOTAL
1	M	60	Chronic myeloid	1	6	7.9	122	27	–	2	16	33	21	1	35	7	42
2	F	36	Chronic myeloid	2	6	8.2	95	30	–	1	14	41	11	3	16	13	29
3	M	63	Chronic lymphatic	3	6.5	10.82	90.5	5	95	–	–	–	–	–	26	12	38
4				4	6.5												
5	M	53	Chronic lymphatic	5	6.5	10.92	95	7	93	–	–						
				6	6	10.86	15	15	85	–	–	–	–	–	11	29	40 Still alive

TABLE III

PROTOCOL	SEX	AGE (YR.)	RECIPIENT'S LEUCEMIA	DONOR'S LEUCEMIA	RECIPIENT'S BLOOD AT TIME OF INOCULATION								DURATION OF RECIPIENT'S LEUCEMIA (MO.)		
					HEMOGLOBIN (GM. %)	W.B.C. 10^3	POLYMORPHONUCLEARS	LYMPHOCYTES	EOSINOPHILES	BASOPHILES	MYELOCYTES	MYELOBLASTS	BEFORE INOCULATION	AFTER INOCULATION	TOTAL
1	M	45	Chronic lymphatic	Chronic myeloid	9.2	11	10	90	–	–	–	–	13	1/52	13 1/52
2	M	53	Chronic lymphatic	Chronic myeloid	10.64	5.3	54	46	–	–	–	–	13	27/12 Still alive	40 1/2
3	F	36	Chronic myeloid	Chronic lymphatic	8.26	51.5	54	–	1	7	31	7	19	11	30
4	M	42	Chronic myeloid	Chronic lymphatic	6.8	68	62	–	–	5	30	3	48	15	63
5	F	32	Chronic myeloid	Chronic lymphatic	9.5	28	45	–	6	7	41	1	19	15 1/2	34 1/2
6	M	36	Chronic myeloid	Chronic lymphatic	7.8	13.1	75	2	2	16	5	1	36	6 1/2	42 1/2

The fate of the recipients and donors is recorded in Tables II and III.

Donors and recipients had deep x-ray and arsenic therapy in the six months following the transmissions according to the state of their health or whenever necessary.

DISCUSSION

Whereas in fowls and mice leucemia can readily be transmitted in susceptible strains and only a minimal amount (one leucemic cell) of leucemic material is necessary for a successful transmission, all attempts of previous workers and the ones described herewith in man have failed. So far no transmission of leucemia from man to man, using blood, lymph nodule, or spleen, has been established. The reason for this failure might be sought in the following factors:

A. (1) In the technique employed. The assumed leucemic agent might also not be able to contact sufficient susceptible cells of its own type, that is, cells of the bone marrow or lymph nodules, by the subcutaneous or intravenous routes which were employed for the attempted transmissions. The agent of human leucemia might be extremely sensitive and not survive the procedure of transmission.

(2) In the material used. Whole blood, lymph nodules, and spleen might not be suitable for transmission in that they might either contain no leucemic agent or have it only in an inactive form.

(3) Time of observation. The time of observation in many cases was certainly very short, but even twenty-seven months might not be sufficient time for the agent to establish itself and produce a leucemia.

(4) The choice of hosts.

(a) As only people with a life prospect of under two years were available and all suffered from incurable diseases, the recipients could not be regarded as "virginal organisms." These patients might have had sufficient antibodies to prevent a take.

(b) The recipient might also have had a natural immunity, might have belonged to a nonsusceptible strain of man, or might have had an acquired immunity protecting him against any leucemic agent.

B. It also might be that human leucemia, contrary to animal leucemia, cannot be transmitted and therefore all attempts were useless from the start.

The fact that even material from acute leucemias would not produce reactions in the recipients is remarkable as one would expect that this type of leucemia with its fulminant course would be the easiest to transmit.

Of all the factors mentioned, the difficulty of obtaining suitable hosts and the apparently unsuitable routes are the most important and may certainly be responsible for the negative results of the transmission attempts.

SUMMARY

1. Attempts were made to transmit leucemia from man to man.

2. Material used consisted of blood and lymph nodular and splenic emulsions of chronic myeloid, lymphatic, and acute leucemias.

3. Attempts were made with whole blood to cross-transmit chronic myeloid leucemia to patients with chronic lymphatic leucemia and *vice versa*.

4. All transmission attempts from man to man and cross-transmissions between patients with leucemia failed with a period of observation of two years.

5. The explanation is regarded to be mainly due to unsuitable recipients and unsuitable route of transmission.

6. The studies brought forward no evidence that human leucemia is transmissible.

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HEMATOLOGIC VALUES OF AMERICAN SOLDIERS STATIONED IN THE TROPICS

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THIS paper is the result of a survey of blood counts on American soldiers who have been stationed on tropical islands for twenty-four months. An unusual opportunity to study one group over a long period was afforded because the majority was from one task force.* The survey was initiated because frequently medical officers would remark that "each month the red blood cell count seems to be lower and the incidence of eosinophilia increased." If this is true, then it would be a significant indication of the health of soldiers who have been garrisoned almost continuously on tropical islands since March, 1942.

MATERIAL AND METHODS

The material for this study was selected at random from the laboratory and clinical records of an Army hospital stationed on islands in the South and Southwest Pacific theaters of war since March, 1942. Data were collected from July, 1942 (six months after the group selected for study had left the continental limits of the United States), to January, 1944. The study was arbitrarily divided into three periods of six months each. Period I includes those studied from July, 1942, to January, 1943; Period II, from January, 1943, to July, 1943; and Period III, from July, 1943, to January, 1944. The same controls were utilized during the three periods. A comparable control group was studied in January, 1945, eleven months after the completion of this report.

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*Two exceptions were (1) an organization that served one and one-half years in Panama and (2) one that served a year on another South Pacific island prior to joining the task force.

The same laboratory technicians performed the blood counts during the entire period of study. The Tallquist hemoglobin scale (15.8 Gm. of hemoglobin per 100 c.c. of blood) was the only method available for determining hemoglobin. An eosinophile count of 9 per cent or more was considered abnormal. All the patients had one or more stool examinations if eosinophilia was present.

The majority of all those studied lived in the northern United States prior to overseas duty. During Period I, all were garrisoned on a large base island in the South Pacific; during Period II, a large portion of patients studied were subjected to severe combat experience; during Period III, all studied were stationed on an isolated island in the Southwest Pacific but were not engaged in combat.

RESULTS

Erythrocyte Counts and Hemoglobin Determination.—During Period I (July, 1942, to January, 1943) the histories of 142 patients who had been stationed in the Tropics for six months were reviewed. The average erythrocyte count was 4,710,000, while the average hemoglobin determination was 88 per cent. Bacillary dysentery and acute infectious hepatitis were the main causes for admission.

During Period II (January, 1943, to July, 1943) the histories of 211 patients who had been stationed in the Tropics twelve months were reviewed. The average erythrocyte count was 4,560,000, and the average hemoglobin determination was 86 per cent. Respiratory diseases, bacillary dysentery, and malaria were the main causes for admission.

During Period III (July, 1943, to January, 1944) the histories of 302 patients who had been stationed in the Tropics eighteen months were reviewed. The average erythrocyte count of the group was 4,390,000, and the average hemoglobin determination was 84 per cent. Filariasis and malaria were the main causes for admission (Table I).

Controls.—A group of healthy soldiers selected from the hospital personnel, who suffered no illness requiring hospitalization during the period of observation, was utilized as controls. Complete blood counts are available on 14, made shortly after they arrived in the Tropics. The average erythrocyte count was 4,970,000, while the average hemoglobin was 95 per cent. Complete blood counts were performed on 19 others after they had been garrisoned in the South Pacific for one year. At that time the average erythrocyte count of the 33 was 4,770,000, while the hemoglobin average was 91 per cent. At the end of two years the average erythrocyte count of the 33 was 4,400,000, with a hemoglobin average of 84 per cent* (Table II).

Anemia.—During Period I, 1 (0.7 per cent) patient of 142 studied was classified as having "borderline" normocytic anemia. No one in the control group was anemic. During Period II, 13 (6.1 per cent) of 211 patients studied were classified as having normocytic anemia. Similar findings were obtained in 2 (6 per cent) of 33 controls. In Period III, 29 (9.6 per cent) of 302 patients were classified as having normocytic anemia, while 3 (9 per cent) of 33 controls had similar findings (Table III).

Leucocyte and Differential Counts.—White blood cell and differential leucocyte counts in bacillary dysentery, acute infectious hepatitis, malaria, dengue,

*At the end of three years in the tropics, a comparable but not identical group of controls had an average erythrocyte count of 4,700,000, while the hemoglobin average was 84 per cent. No one in the group was anemic.

TABLE I. AVERAGE ERYTHROCYTE COUNTS AND HEMOGLOBIN DETERMINATIONS ON PATIENTS STATIONED IN THE TROPICS FROM JULY, 1942, TO JANUARY, 1944

PERIOD	DISEASE	NUMBER OF CASES STUDIED	R.B.C. (IN MILLIONS)	HB. (%)	PER CENT* OF GROUP
Period I (Patients in Tropics from 6 to 12 months)	Dysentery, bacillary	23	4.69	86.9	16.2
	Peptic ulcer	8	4.68	88.8	5.6
	Hepatitis, infectious	28	4.96	88.2	20.0
	Neurosis (gastric, cardiac)	13	4.64	86.9	9.1
	Malaria, primary attack	13	4.49	86.1	9.1
	Respiratory (atypical pneumonia, bronchitis)	9	4.63	88.3	6.3
	Allergic (asthma, dermatoses)	11	4.72	88.5	7.7
	Arthritis	5	4.28	86.0	3.5
	Miscellaneous (including surgical cases)	26	4.78	92.1	18.3
	No disease found	6	4.45	82.5	4.2
	Summary	142	4.71 Av.	88.0 Av.	100.0
Period II (Patients in Tropics from 12 to 18 months)	Dysentery, bacillary	23	4.63	84.4	10.9
	Peptic ulcer	12	4.53	85.4	5.7
	Neurosis (gastric, cardiac)	14	4.66	87.5	6.6
	Malaria, recurrent	20	4.69	86.7	9.5
	Respiratory (atypical pneumonia, bronchitis)	29	4.64	86.7	13.7
	Allergic (asthma, dermatoses)	16	4.75	89.6	7.6
	Intestinal parasites (hookworm, whipworm)	10	3.89	81.5	4.7
	Dengue	4	4.70	86.2	2.0
	Renal (pyelo-nephritis, lithiasis)	16	4.55	80.9	7.6
	Miscellaneous (including surgical cases)	57	4.47	87.6	27.0
	No disease found	10	4.73	90.5	4.7
	Summary	211	4.56 Av.	86.0 Av.	100.0
Period III (Patients in Tropics from 18 to 24 months)	Dysentery, bacillary	2	4.50	82.5	.7
	Peptic ulcer	3	4.56	80.0	1.0
	Neurosis (gastric, cardiac)	11	4.30	81.8	3.6
	Malaria, primary attack	16	4.54	34.6	5.3
	Malaria, recurrent attack	13	4.42	85.0	4.3
	Respiratory (atypical pneumonia, bronchitis)	4	4.12	76.2	1.3
	Allergic (dermatoses, intestinal parasites)	9	4.27	78.8	3.0
	Filariasis	70	4.31	84.2	23.2
	Arthritis	2	4.20	80.0	.6
	Miscellaneous (including surgical cases)	124	4.46	84.8	41.1
	No disease found	48	4.35	32.9	15.9
	Summary	302	4.39 Av.	84.0 Av.	100.0

*For statistical reasons average red blood cell counts and hemoglobin determinations for each period are calculated on a percentage basis.

dermatoses, intestinal helminthiasis, and filariasis—diseases commonly encountered in these theaters—did not differ from established values and are not presented.

Eosinophilia.—During Period I, of 142 patients studied, 1 (0.7 per cent) with hookworm disease had an eosinophile count of 9 per cent. During Period II, of 211 patients studied, 7 (3.3 per cent) had an eosinophile count of 9 per cent or more. Eosinophilia was present in two patients with hookworm disease and in one with periarteritis nodosa. It was unexplained in 4 patients. During Period III, of 256 patients studied, 26 (10.1 per cent) had an eosinophilia. Eosinophilia was present in patients with malaria, dermatoses, intestinal helminthiasis, and filariasis. Eosinophilia was unexplained in 9 patients (Table IV).

TABLE II. ERYTHROCYTE AND HEMOGLOBIN DETERMINATIONS ON HEALTHY SOLDIERS GARRISONED IN THE TROPICS OVER A PERIOD OF TWO YEARS

	SIX MONTHS OR LESS IN TROPICS		ONE YEAR IN TROPICS		TWO YEARS IN TROPICS	
CASE	R.B.C. (IN MILLIONS)	HB. (PER CENT)	R.B.C. (IN MILLIONS)	HB. (PER CENT)	R.B.C. (IN MILLIONS)	HB. (PER CENT)
1	5.0	95	4.7	85	4.8	100
2	5.0	100	3.8*	65*	4.0	80
3	5.0	90	4.9	90	4.1	80
4	4.7	90	4.1	80	3.7*	80*
5	4.7	90	4.5	95	4.5	85
6	5.0	100	5.0	95	4.2	85
7	5.0	100	5.2	100	4.5	80
8	4.8	90	5.0	100	5.0	90
9	4.9	95	5.0	95	4.7	85
10	4.9	100	4.6	90	4.4	85
11	5.0	100	5.0	90	4.3	90
12	5.0	90	4.2	85	3.8*	70*
13	5.2	100	3.7*	65*	4.3	85
14	5.5	100	5.7	100	4.1	90
15			4.7	90	4.8	90
16			4.0	85	4.7	85
17			4.6	95	4.4	90
18			4.7	95	4.5	85
19			4.4	85	3.9*	70*
20			5.0	95	4.2	85
21			5.2	100	4.5	80
22			5.0	100	5.0	90
23			5.0	95	4.7	85
24			4.6	90	4.4	85
25			5.0	90	4.3	90
26			5.7	100	4.1	90
27			4.8	95	4.3*	75*
28			5.3	100	4.3	80
29			4.7	90	4.8	85
30			4.7	90	4.5	90
31			4.9	95	4.2*	75*
32			5.1	100	4.9	100
33			4.9	90	4.4	85
TOTAL			TOTAL		TOTAL	
14	4.97	95	33 4.77	91	33 4.40	84

*Regarded as having an anemia. No organic reason could be found.

In order to clarify the higher incidence of eosinophilia during Period III, those studied during that period were divided into two groups: (A) those who had been garrisoned on filarial-free islands and (B) those who had been stationed for one year prior to joining the task force on an island where filariasis was endemic. (Previous observations on soldiers with filariasis[1] led us to expect a higher incidence of eosinophilia in that group.) Of 100 patients in Group A, 8 (8 per cent) had eosinophilia, while of 156 in Group B, 18 (11.5 per cent) had similar findings. Patients with eosinophilia in Group A had malaria, dermatoses, or hookwork infection, while in Group B, except for filariasis, eosinophilia could not be explained. These patients were admitted for inguinal hernia, impact molars, penile ulcer, acute infectious hepatitis, otitis externa, pharyngitis, cellulitis, and furunculosis.

Differential leucocyte counts on 282 healthy soldiers were utilized as controls. These men were also divided into two groups: (C) those garrisoned on filarial-free islands and (D) those exposed to filariasis. In Group C, 5 (3.5 per cent) of 140 had eosinophilia,* while 40 (28.1 per cent) of 142 in Group D

*During January, 1945, eleven months after leaving the island where the hospital was stationed during Period III, eosinophile counts were performed on 70 healthy soldiers, all from the original Group C. These men were all hospital personnel who had come overseas as a unit in March, 1942. They had been garrisoned almost continuously on tropical islands since that time. The study was important because it was now definitely known that the control group had lived in proximity to the organizations exposed en masse to filariasis on another island in 1943.[4] Of 68 controls, 5 (7.3 per cent) had an eosinophilia of 9 per cent or more: 2 had hookworm disease, and 1 had eczematoid dermatitis, while in 2 the eosinophilia was unexplained even after careful investigative studies. They did not have signs or symptoms of filariasis.

TABLE III. ANEMIA IN SOLDIERS STATIONED IN THE TROPICS

PATIENTS					CONTROLS						
PERIOD	TOTAL CASES	NUMBER WITH ANEMIA	INCIDENCE (PER CENT)	DISEASE	R.B.C. (IN MILLIONS)	HB. (PER CENT)	TOTAL CASES	NUMBER WITH ANEMIA	INCIDENCE (PER CENT)	R.B.C. (IN MILLIONS)	HB. (PER CENT)
I (July-Jan., 1943)	142	1	0.7	No disease found	4.0	70	14	0	0	--	--
II (Jan.-July, 1043)	211	13	6.1	Dysentery, bacillary	3.7	65	33	2	6	3.8	65
				Malaria, recurrent	3.5	60				3.9	65
				Amebiasis	4.1	65					
				Varicose veins	3.0	75					
				Hookworm	3.4	75					
				Hookworm	3.4	75					
				Hookworm	3.7	80					
				Hookworm	3.7	80					
				Dysentery, bacillary	3.7	65					
				Dysentery, bacillary	4.1	65					
				Neurasthenia	4.1	65					
				Malaria, recurrent	3.5	60					
				Appendicitis	3.7	65					
III (July, 1943-Jan., 1944)	302	29	9.6	Neurasthenia	3.6	75	33	3	9	3.7	80
				Neurasthenia	3.9	75				3.8	70
				Neurasthenia	3.6	75					
				Malaria, primary	3.9	70				3.9	70
				Malaria, recurrent	3.1	55					
				Malaria, recurrent	3.6	70					
				Malaria, recurrent	3.9	75					
				Dermatoses	3.9	70					
				Dermatoses	3.7	70					
				Dermatoses	3.8	75					
				Dermatoses	3.5	70					
				Respiratory	3.9	70					
				Respiratory	3.7	70					
				Respiratory	3.9	70					
				Anemia	3.7	75					
				Myositis	4.0	70					
				Otitis media	3.6	70					
				Hepatitis	3.9	75					
				Fracture	3.8	75					
				No disease found	3.6	75					
				No disease found	3.6	75					
				No disease found	4.1	60					
				No disease found	4.0	75					
				No disease found	3.6	75					
				No disease found	3.4	65					
				No disease found	3.7	75					
				No disease found	3.6	65					
				No disease found	3.8	75					
				No disease found	3.9	70					

TABLE IV. INCIDENCE OF EOSINOPHILIA (9 PER CENT OR MORE EOSINOPHILES) IN AMERICAN SOLDIERS IN THE TROPICS

PERIOD	TOTAL CASES	TOTAL CASES WITH EOSINOPHILIA	INCIDENCE EOSINOPHILIA (PER CENT)	DISEASE	NUMBER OF CASES	NUMBER OF CASES WITH EOSINOPHILIA
I	142	1	0.7	Hookworm	1	1
II	211	7	3.3	Periarteritis nodosa	1	1
				Hookworm	15	2
				Miscellaneous	18	4
III	256	26	10.1	Malaria, primary	16	2
				Malaria, recurrent	13	2
				Dermatoses	6	1
				Hookworm	3	2
				Filariasis	70	10
				Miscellaneous	148	9

had similar findings. In summation, 13 (5.4 per cent) of 240 soldiers (patients and controls) not exposed to filariasis had eosinophilia compared with 58 (19.4 per cent) of 298 who were exposed to the disease (Table V).

TABLE V. EOSINOPHILIA IN AMERICAN SOLDIERS EXPOSED TO FILARIASIS COMPARED TO THOSE NOT EXPOSED

PERIOD III				
GROUP	DISEASE	NUMBER OF CASES	NUMBER OF CASES WITH EOSINOPHILIA	INCIDENCE (PER CENT)
A. Patients never exposed to filariasis	Malaria, primary	16	2	
	Malaria, recurrent	13	2	
	Dermatoses	6	1	
	Hookworm disease	3	2	
	Miscellaneous*	62	1	
Total		100	8	8.0
B. Patients exposed to filariasis	Filariasis, clinical	70	10	
	Miscellaneous†	86	8	
Total		156	18	11.5
C. Healthy soldiers not exposed to filariasis		140	5	3.5
D. Healthy soldiers exposed to filariasis	No clinical signs	142	40	28.1
Total				
Those not exposed to filariasis		240	13	5.4
Those exposed to filariasis		298	58	19.4

*No disease found.

†Inguinal hernia, impacted molars, penile ulcer, acute infectious hepatitis, otitis externa, pharyngitis, cellulitis, furunculosis.

DISCUSSION

It is generally believed that in the Tropics one may expect to develop anemia. The reasons usually ascribed are vague and the term "physiologic anemia" is used. Except for the high incidence of anemia due to parasitic infestations and dietary deficiency, the causes for this finding in indigent populations are similar to those which occur in persons living in a temperate climate. It is difficult to conceive that the causes for anemia in American soldiers in the Tropics would be comparable with indigents because of entirely different dietary and sanitary habits. Still, this report indicates that erythrocyte and hemoglobin values on soldiers who have been garrisoned continually in the Tropics over a two-year period are below the stated normal limits for young healthy males.[2] Although red blood cell counts are lower than normal, the actual incidence of anemia is negligible. It is important to determine if these findings are significant.

There are several possible explanations for lowered erythrocyte-hemoglobin values, such as chronic blood loss, iron deficiency, nutritional deficiency, alterations in the physiologic state of the body, or normal error in the erythrocyte count. There is increasing evidence that a diet deficient in iron does not produce iron-deficient anemia except in the presence of increased needs for iron, such as growth, pregnancy, or blood loss. In the Tropics iron-deficient anemia could be due to diminished or defective gastric secretions, but Heath[3] emphatically states that although inadequate iron absorption may be a complicating factor, blood loss in a subtle or chronic form can always be demonstrated. Malaria, amebiasis, hookworm disease, and the enteritides, diseases which represent a good portion of our study, are known to be associated with blood loss.

Lack of thiamine, riboflavin, and ascorbic acid may be contributing factors to defective blood formation.[4]

Statistically, the data accumulated on the patient group are not significant, since the number of cases studied is small and not identical in each period, and the severity of illnesses is not known to be comparable. It is assumed that the anemias reported in this group were secondary to the diseases. However, the data collected on the control group are significant. At the end of two years of tropical service, 18 (54.5 per cent) of 33 healthy soldiers had a substantial reduction in erythrocyte and hemoglobin values. These men were carefully studied, but no organic basis could be found for the low values. Rush[5] selected 14 of this group (Cases 1 to 14, Table II) and carefully studied their dietary habits. He found that the diet as served was adequate, but over 90 per cent of the group were eating meals inadequate in one or more essential food elements in that primary dietary deficiencies of calcium, thiamine, riboflavin, and ascorbic acid were revealed. On the other hand, iron and protein ingestion was adequate. All had weight loss during a six-month period (July, 1943, to January, 1944) and 12 had a reduction in red blood cells and hemoglobin. Specific causes for the loss or decrease in hematologic values were lacking except on a nutritional basis. Many possible factors seemed contributory. The most important perhaps was the monotony and lack of palatability of the food. Many complained of loss of energy, sleeplessness, and irritability (common complaints of soldiers in the Tropics) which could be explained by the continued threat of enemy bombing, heat and humidity of the jungle, isolation from civilization, and homesickness. At least one multivitamin tablet a day was added to the diet of these men, but there was no apparent increase either in weight or erythrocyte values during their stay in the jungle.

This same group of men moved to another island in March, 1944, where living conditions and food were much better. In addition, they all had a furlough to Australia. In a study of a comparable (but not identical) group in January, 1945 (thirty-six months of almost continuous tropical service), the average erythrocyte count was 4,700,000 and the average hemoglobin determination was 84 per cent. The majority had returned to normal weight, and their complaints of the previous year were long forgotten. However, although their erythrocyte counts approximated normal, the hemoglobin values did not change. It seems as though a multiplicity of secondary factors which contribute to poor nutrition may be one reason for the "low normal" erythrocyte values. Quinacrine hydrochlorida (atabrine) taken in suppressive doses since June, 1943, may be contributory. Physiologic changes that occur in the Tropics, such as those which increase blood volume, could be the prime factor. It has not been possible in this study to find any specific cause for our observations in healthy soldiers.

If the reasons postulated are true, then our data on patients gain more significance. As a broad general rule, soldiers have not developed anemia in diseases one commonly expects them to. Undoubtedly, early and specific therapy in malaria, helminthiasis, and gastrointestinal disorders minimize blood loss. This observation has been repeatedly checked during the past three years of tropical service. Many patients with these diseases have had several blood counts performed at intervals during their hospitalization. Although severe anemias have been found, especially in those with malaria due to *Plasmodium falciparum*, the data collected convinces one that anemias are not the rule. It seems plausible to conclude that the same factors affecting the control group

affect the patient group. More detailed study of larger control groups will be necessary before any accurate conclusions can be reached concerning specific causes for the observation reported in this paper.

The increasing incidence of eosinophilia presents an interesting problem because it is in the allergic and nonbacterial diseases that eosinophilia occurs. After troops have been stationed in the Tropics for two years, the incidence of eosinophilia (9 per cent or more) was 10.1 per cent compared with an incidence of 4.4 per cent in ambulatory patients studied at the Indianapolis City Hospital.[7] Data comparable with ours are reported by Allen,[8] who studied patients admitted to a Navy hospital in the South Pacific. He felt the increasing incidence was due to hookworm disease. The main reason for the increase of eosinophilia in our study was filariasis. This is best exemplified in the group of healthy soldiers studied during Period III (Table V). Although the group exposed to filariasis had no evidence of the disease, the incidence of eosinophilia was significantly increased over those not exposed.

Unexplained eosinophilia is not an infrequent finding in soldiers stationed in the Tropics. Allen[8] feels that in most instances, if the patient with eosinophilia is studied long enough, a parasitic infection is usually found. Because infestations are usually slight, manifestations may not appear for long periods of time, although eosinophilia, an allergic manifestation, develops early. Since Allen's report was read, 272 patients with an eosinophilia of 9 per cent or more have been carefully studied. Statistical data are not included in this report because groups are not comparable in that the majority spent less time in the Tropics and were garrisoned in Australia between combat missions. Although they were hospitalized for a wide variety of reasons (traumatic wounds, psychoneuroses, gastrointestinal complaints, arthritis, etc.), 55 were found to have helminthiasis, and in 52 others, symptoms were suggestive, but the patients were evacuated to rear areas before they could be studied adequately. Detailed data will be reported at a later date. In our control group of hospital personnel who had been in the Tropics thirty-six months, who have been garrisoned on an island where helminthiasis was hyperendemic in Periods I and II, and who were possibly exposed to filariasis during Period III, only 5 (7.3 per cent) had an eosinophilia, the causes for which were evident in 60 per cent.

SUMMARY AND CONCLUSIONS

1. Blood counts are reported on 655 American soldiers who were admitted to an Army hospital in the South and Southwest Pacific theaters of war.

2. The study was initiated after troops had been garrisoned in the Tropics at least six months and was divided into three six-month periods. Control groups of healthy soldiers were utilized.

3. A slight but progressive decrease below erythrocyte and hemoglobin values usually considered normal for young healthy males occurred. The incidence of true anemia was slight, even in those having diseases in which anemia is commonly found.

4. Possible reasons for these findings are discussed, but specific evidence for low normal values is lacking. Conditions which occurred on an isolated post in a forward area seemed to have played an important role.

5. A marked increase in the incidence of eosinophilia has been noted. The main causes are parasitic infestations and dermatoses.

6. A high incidence of eosinophilia was found in healthy soldiers who were exposed to filariasis but had no clinical evidence of the disease. This finding is

significant because it suggests that many soldiers are being exposed to diseases in which allergy may be an important factor. Because of minimal infestation, symptoms usually ascribed to these diseases may never become manifest.

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LABORATORY METHODS

THE INHIBITION ZONE TECHNIQUE FOR DETERMINATION OF BACTERIAL RESISTANCE TO PENICILLIN

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PENICILLIN assay by the agar plate method is based on the principle that if a stipulated volume of penicillin is placed on the surface of an agar plate seeded with a susceptible organism, the zone (area) of growth inhibition will be a direct function of the concentration of penicillin employed. The cup methods of Abraham and co-workers[1] and of Foster and Woodruff,[2] the filter disk methods of Vincent and Vincent[5] and of Sherwood, Falco, and deBeer,[3] and the loop technique of Thomas, Levine, and Vitagliano[4] are examples of the application of this principle. In penicillin assay, the test organism and volume of penicillin solution applied to the surface of a seeded plate are maintained constant, the concentration of penicillin being the variable to be measured. In a previous communication we have pointed out that if both the volume and concentration of penicillin applied are maintained constant, while the organism employed as a seed is varied, the growth inhibition zone produced will be an inverse function of the resistance of the test organism. It was suggested that such a procedure would be more convenient and expeditious than the rather cumbersome serial dilution methods for evaluating bacterial resistance to penicillin. Responses to these suggestions indicate the desirability for elaboration upon the basis of the proposed technique and applications in the clinical laboratory.

RELATION OF RESISTANCE OF AN ORGANISM TO PENICILLIN IN BROTH AND ZONES OF GROWTH INHIBITION OF AGAR

For the purpose of the following report, resistance of test organisms (staphylococci) is expressed in terms of the minimum concentration of penicillin employed which prevented growth and the maximum concentration which permitted growth, in beef extract broth, after twenty-four hours at 37° C. Thus, if an organism grew in broth containing 0.02, but not if 0.04 Oxford unit of penicillin was present per cubic centimeter of medium, its resistance is expressed as 0.02 unit positive, 0.04 unit negative.

Organisms.—The cultures employed consisted of staphylococci obtained from suppurating infections and variants of the standard Oxford strain of *Staphylococcus aureus,* which were induced to develop increased resistance to penicillin, by serial transfer in broth containing progressively increasing concentrations of penicillin. (From a strain of *Staph. aureus* which did not grow in 0.04 Oxford unit per cubic centimeter, cultures which grew in broth containing 0.04 to 2.5 Oxford units per cubic centimeter were obtained after from two to fifteen test-tube generations.)

From the Clinical Laboratory Service, Brooke General Hospital, Fort Sam Houston, Texas. Received for publication, July 20, 1945.

Penicillin.—The penicillin solutions employed for this study were those encountered in routine clinical work diluted to make concentrations of 10, 5, 2.5, 1.25, and 0.63 Oxford units per cubic centimeter in phosphate buffer (M/50 at pH 7.2). All of these penicillin samples, when diluted serially in broth, markedly inhibited growth of the Oxford strain of *Staph. aureus* in a concentration of 0.02 Oxford unit per cubic centimeter of medium, as did also a secondary penicillin standard. When 4 mm. loopfuls of these penicillin solutions containing 10 Oxford units per cubic centimeter were placed on agar plates seeded with the Oxford strain of *Staph. aureus*, growth inhibition zones of 29 ± 1 mm. in diameter were produced. Comparison with a secondary penicillin standard * disclosed that all samples were within limits of error inherent in penicillin assays and the leeway (± 15 per cent) permitted in army specifications.

Preparation of Plates.—Seeded agar plates were prepared by inoculating 0.05 c.c. of a 20 to twenty-four hour broth culture of a test organism into 15 c.c. of melted, cooled (from 42 to 48° C.) agar which was then poured into a Petri dish which, immediately after hardening, was placed in the refrigerator (from 5 to 10° C.) where it was kept until ready for use (from two to eight hours).

Determination of Growth Inhibition Zones.—A 4 mm. loopful of each of the aforementioned concentrations of penicillin was placed on the surface of a chilled agar plate which had been seeded with staphylococci of various degrees of resistance (as determined by growth in broth containing known concentrations of penicillin), and the plates were immediately placed in the incubator at 37° C., taking care to maintain the plates horizontal in order to insure development of circular growth inhibition zones. After from eighteen to twenty-two hours' incubation, the diameters of the growth inhibition zones were measured to the nearest 0.5 mm.

Determination of Inhibiting Concentration of Penicillin in Broth.—Beef extract broth containing various concentrations of penicillin (2.5, 1.25, 0.63, 0.32, 0.16, 0.08, 0.04, 0.02, and none) was distributed aseptically into test tubes (10 c.c. quantities). A tube of each concentration was inoculated with a drop (0.05 c.c.) of a (twenty to twenty-four hours at 37° C.) broth culture of the test organism. The inoculated tubes were then placed at 37° C. and vigor of growth recorded after from eighteen to twenty-two hours' incubation.

TABLE I. GROWTH INHIBITION ZONES PRODUCED BY VARIOUS CONCENTRATIONS OF PENICILLIN ON AGAR SEEDED WITH STRAINS OF STAPHYLOCOCCI OF VARYING DEGREES OF RESISTANCE TO PENICILLIN IN BROTH

GROUP	NUMBER OF STRAINS	CONCENTRATION OF PENICILLIN IN BROTH (OXFORD UNITS PER C.C.)		CONCENTRATION OF PENICILLIN APPLIED (4 MM. LOOPFULS) TO SEEDED AGAR (OXFORD UNITS PER C.C.)				
		PERMITTING GROWTH (24 HR.)	PREVENTING GROWTH (24 HR.)	10	5	2.5	1.25	0.63
				ZONE OF GROWTH INHIBITION (DIAMETER IN MM.)				
A	16	0.02	0.04	28.9 ± 1.2	24.6 ± 0.8	20.4 ± 1.1	15.0 ± 2.5	10.5 ± 2.9
B	9	0.04	0.08	24.6 ± 1.3	21.3 ± 1.2	16.9 ± 1.3	12.0 ± 2.0	< 5*
C	7	0.08	0.16	18.9 ± 1.0	15.2 ± 1.1	11.6 ± 1.3	< 5*	
D	4	0.16	0.32	17.1 ± 0.3	12.3 ± 0.9	< 5*		
E	5	0.32	0.63	14.0 ± 0.8	9.9 ± 0.7			

*Inhibition zone not distinct.

*Supplied by Merck & Co. Inc., Rahway, N. J.

Comparison of Inhibiting Concentration in Broth With Inhibition Zone on Agar.—The results obtained with the foregoing techniques for measuring bacterial resistance to penicillin are summarized in Table I, where (1) the test organisms are allocated to five groups on the basis of the maximum concentration of penicillin permitting and the minimum concentration preventing growth in broth and (2) the diameters of the growth inhibition zones (with their standard deviations) obtained on agar seeded with organisms of the respective groups are shown.

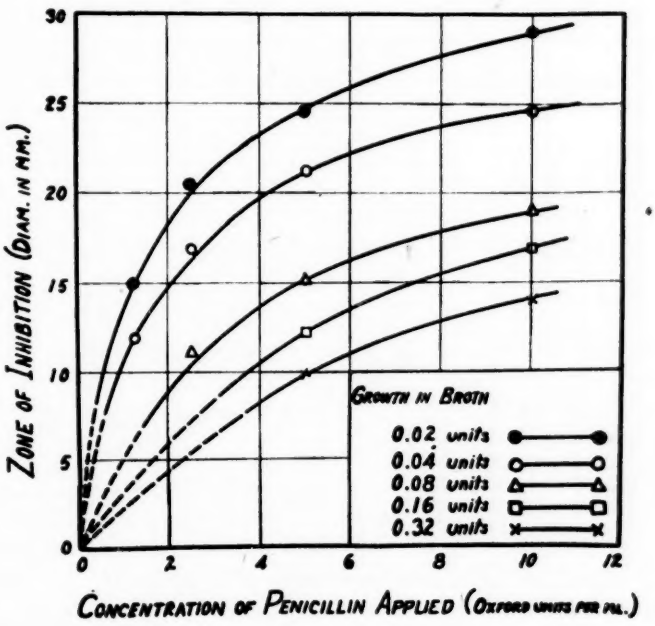

Fig. 1.—Relation of concentration of penicillin to inhibition zones produced against staphylococci of various degrees of resistance to penicillin in broth.

By plotting the inhibition zones against the concentrations of penicillin applied, the curves shown in Fig. 1 are obtained. It will be noted from Table I that when concentrations of penicillin of less than 2.5 Oxford units per cubic centimeter were used, the diameters of inhibition zones obtained showed a rather high degree of variability, a phenomenon which is generally the case for inhibition zones of less than 12 mm. diameter. It will be further noted that when employing a penicillin solution containing 2.5 Oxford units per cubic centimeter, the inhibition zones produced with organisms of group D (growth in broth containing 0.16 but not if 0.32 Oxford unit of penicillin per cubic centimeter is present) which are probably at the upper limit of susceptibility with present methods of systemic penicillin treatment, the inhibition zones are too small for satisfactory evaluation. Even for organisms of group C (growth in broth with 0.08 but not 0.16 Oxford unit of penicillin per cubic centimeter), which are quite susceptible, the inhibition zones produced with a loop of penicillin solution containing 2.5 Oxford units per cubic centimeter are in the range of minimum reliability. It is therefore suggested that penicillin solutions containing 10 or 5 Oxford units per cubic centimeter would be most useful for rapidly determining penicillin sensitivity by the method of ascertaining the growth inhibition zones produced on agar plates seeded with a test organism.

If the growth inhibition zones obtained for the various groups of test organisms (employing a standard 4 mm. loop of 10 or 5 Oxford units per cubic centimeter of buffered penicillin solution) are plotted against the resistance of the respective groups as shown by growth in broth with various concentrations of penicillin (for example, the maximum concentrations of penicillin employed which permitted, and the minimum concentrations which prevented, growth in broth), the curves in Fig. 2 are obtained. Table II was then developed from these curves (Fig. 2) and shows the probable concentrations of penicillin (expressed in Oxford units per cubic centimeter) which would permit or prevent growth in broth, for designated inhibition zones obtained on an agar plate seeded with a test organism.

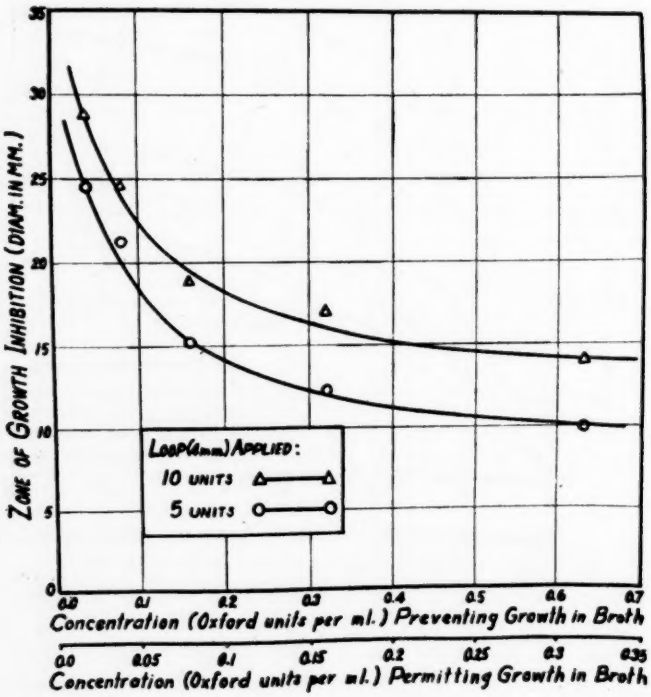
Zone of Growth Inhibition (Diam. in Mm.)
Loop(4mm) Applied:
10 units
5 units
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7
Concentration (Oxford units per ml.) Preventing Growth in Broth
0.0 0.05 0.1 0.15 0.2 0.25 0.3 0.35
Concentration (Oxford units per ml.) Permitting Growth in Broth

Fig. 2.—Relation of inhibition zones on seeded agar to resistance of staphylococci to penicillin in broth.

In practice, knowledge of the exact degree of resistance of an organism to penicillin is not so significant as is the information as to whether a suspected organism is so susceptible (or resistant) that the concentration of penicillin maintainable in the blood stream may reasonably be expected to be effective (or ineffective) against the organism in question. A concentration of 0.25 Oxford unit per cubic centimeter of blood is maintainable by the intravenous drip and intramuscular administration of penicillin. Assuming that an organism which grows in broth containing 0.2 Oxford unit, but not in 0.4 Oxford unit, of penicillin per cubic centimeter is near the limit of bacterial resistance amenable to the present available methods for systemic treatment with penicillin, then, if a 4 mm. loopful of a solution containing 10 Oxford units of penicillin per cubic centimeter were applied to a plate seeded with the organism in question, the growth inhibition zone produced would be 15 mm. in diameter. (If the penicillin solution employed for the test contained 5 Oxford units per cubic centimeter, an inhibition zone of 11 mm. would be obtained [see Table II].)

TABLE II. RELATION OF GROWTH INHIBITION ZONE ON AGAR SEEDED WITH STAPHYLOCOCCI TO PROBABLE CONCENTRATION OF PENICILLIN PERMITTING AND PREVENTING GROWTH IN BROTH

10 OXFORD UNITS PER C.C. (4 MM. LOOPFULS APPLIED TO SURFACE OF SEEDED AGAR)			5 OXFORD UNITS PER C.C. (4 MM. LOOPFULS APPLIED TO SURFACE OF SEEDED AGAR)		
ZONE OF GROWTH INHIBITION ON SEEDED AGAR PLATE (IN MM.)	PROBABLE CONCENTRATIONS* IN BEEF EXTRACT BROTH (OXFORD UNITS PER C.C.)		ZONE OF GROWTH INHIBITION ON SEEDED AGAR PLATE (IN MM.)	PROBABLE CONCENTRATIONS* IN BEEF EXTRACT BROTH (OXFORD UNITS PER C.C.)	
	PERMITTING GROWTH	PREVENTING GROWTH		PERMITTING GROWTH	PREVENTING GROWTH
30	0.02	0.03	. . . .	. . . .	. . . .
29	0.02	0.04	. . . .	. . . .	. . . .
28	0.03	0.05	28	0.01	0.02
27	0.03	0.05	27	0.02	0.03
26	0.03	0.06	26	0.02	0.03
25	0.04	0.07	25	0.02	0.04
24	0.04	0.08	24	0.03	0.05
23	0.05	0.10	23	0.03	0.06
22	0.06	0.12	22	0.03	0.06
21	0.07	0.13	21	0.04	0.07
20	0.09	0.15	20	0.04	0.08
19	0.08	0.18	19	0.05	0.09
18	0.11	0.22	18	0.06	0.11
17	0.14	0.27	17	0.06	0.12
16	0.16	0.32	16	0.07	0.14
15	0.21	0.42	15	0.09	0.17
14	0.33	0.65	14	0.11	0.21
13	. . . .	. . . .	13	0.13	0.25
12	. . . .	. . . .	12	0.16	0.32
11	. . . .	. . . .	11	0.21	0.42
10	. . . .	. . . .	10	0.32	0.63

*To nearest 0.01 unit.

Our experience has indicated that for streptococci (and other bacteria which do not grow readily on plain agar but grow well on blood media), determination of growth inhibition zones on seeded blood agar plates yielded very satisfactory results. When employing such plates with hemolytic (alpha and beta) streptococci or staphylococci, the area of growth inhibition is indicated by a zone of unhemolyzed red cells surrounded by a narrow clear hemolyzed zone due to diffusion of hemolytic agents from the vicinity of bacterial growth. With the strains of pneumococci encountered, and employing a heavy inoculum, the growth inhibition zone consisted of a series of concentrically ringed bands producing a rainbowlike effect—an area of unhemolyzed red cells surrounded by a rather wide zone of light greening and hemolysis which was, in turn, circumscribed by a narrow zone of what appeared to be heavy growth. With a light inoculum this rainbowlike effect was not manifest.

In general, if the growth inhibition zone produced by applying a 4 mm. loopful of a penicillin solution containing 10 Oxford units per cubic centimeter to the surface of a seeded agar plate is less than 10 mm. in diameter, the test organism would be considered too resistant to anticipate satisfactory results; inhibition zones of from 10 to 15 mm. in diameter would place the organism in a questionable category regarding amenability to systemic penicillin therapy; organisms showing inhibition zones greater than 15 mm. in diameter would be considered sufficiently susceptible to warrant expectation of satisfactory results with the present methods of systemic penicillin therapy. If, in the future, higher penicillin blood levels can be maintained than are now feasible, it will merely be necessary to increase, appropriately, the concentration of the test solution (10 Oxford units per cubic centimeter as now recommended) to adapt the proposed method to the new conditions.

The inhibition zone technique described herein for determination of bacterial resistance to penicillin is subject to less error, requires no special apparatus and considerably less media and equipment, and is not subject to the hazard of contamination associated with the various dilution techniques; the probable degree of resistance can, if necessary, be tentatively determined within eight to ten hours after application of penicillin to seeded plates.

PROCEDURE FOR DETERMINATION OF BACTERIAL RESISTANCE TO PENICILLIN BY THE LOOP GROWTH INHIBITION ZONE TECHNIQUE

A. Materials needed

1. Broth (or other liquid medium) culture of test organism (eighteen to twenty-four hours at 37° C.)
2. Melted agar (15 c.c.) cooled to from 42 to 48° C.
3. One sterile Petri dish (marked off in quadrants)
4. Pipettes—1, 5, and 10 c.c. capacity
5. Standard 4 mm. loop (preferably platinum)
6. M/50 phosphate buffer at pH 7.2 (may be prepared by adding 27 c.c. KH_2PO_4 solution containing 2.72 Gm. per liter to 73 c.c. Na_2HPO_4 solution containing 2.84 Gm. per liter)
7. Penicillin solution (10 and/or 5 Oxford units per cubic centimeter in M/50 phosphate buffer)
8. Sterile flasks (150 c.c.) and test tubes (about 18 mm. diameter)

B. Procedure

1. Preparation of penicillin solutions
   (a) Place 1 c.c. of penicillin (as prepared for therapeutic use and presumably containing 5,000 Oxford units per cubic centimeter) into 49 c.c. of the phosphate buffer (this will make a solution of 100 Oxford units per cubic centimeter).
   (b) Add 1 c.c. of this diluted penicillin to 9 c.c. phosphate buffer to prepare a solution containing 10 Oxford units per cubic centimeter. (If desired, a solution of 5 Oxford units may be prepared by mixing equal volumes of this solution with phosphate buffer.)

   Note: In view of the error inherent in penicillin assay and unavailability of penicillin standards for clinical laboratories, penicillin (5,000 Oxford units per cubic centimeter) solutions employed (diluted 1:500 for 10 or 1:1000 for 5 Oxford units per cubic centimeter) should be tested as described below against the Oxford strain of *Staph. aureus* (or other suitable organism). A growth inhibition zone of 29 ± 1 mm. in diameter with a dilution of 10 Oxford units (or 25 ± 1 mm. with that of 5 Oxford units) may be taken to indicate that the penicillin solution is within the limits of concentration usable for determination of bacterial resistance.

2. Preparation of seeded agar plates

   Add 0.05 c.c. (or one drop from a 1 c.c. pipette) of a broth culture (from 20 to twenty-four hours at 37° C.) of the test organism (Staphylococcus*) to 15 c.c. of melted, cooled (from 42 to 48° C.) agar. Roll between the palms of the hands to distribute the inoculum (but avoid bubbles if possible) and pour into a Petri dish. Mix, permit to solid-

*For streptococci (or other organisms which do not grow readily on nutrient agar but grow luxuriantly on blood media), add from 0.5 to 0.75 c.c. of citrated blood before introducing the test organism, and it may be desirable to increase the size of the inoculum to 0.1 c.c. of a liquid medium in which the test organism grows well.

ify, and place in the refrigerator just as soon after solidification as is feasible. (Seeded plates prepared in this manner may be used after from two to eight hours' cooling, and plates kept in the refrigerator for twenty-four hours have given satisfactory results.)

3. Determination of resistance (growth inhibition zone).

(a) Remove the cooled, seeded agar plate from the refrigerator and

(1) Wipe off any moisture appearing on the inner surface of the Petri dish lid with a clean towel or cloth.

Note: There were no difficulties due to moisture accumulating on the upper lid of the Petri dish, when maintained right side up in water-jacketed incubators, and only three instances of inconsequential contamination have been noted among over 4,000 bacterial resistance tests, when the dishes were wiped as described above.

(2) Mark off the bottom of the plate into four sectors (if not already so marked) and label for identification.

(b) Place a 4 mm. loopful of a penicillin solution containing 10 Oxford units per cubic centimeter on each of two or more of the sectors. (The practice in this laboratory has been to employ solutions of both 10 and 5 Oxford units per cubic centimeter of penicillin, placing a loop of each of these on each of two quadrants, to provide a check on both the concentration and constancy of loopfuls.)

Note: In our experience, employing a loop (bent at right angles to the wire) inserted into the penicillin solution (preferably in a tube not less than 18 mm. diameter), maintaining the plane of the loop approximately parallel to the surface of the liquid and quickly withdrawing it, in order to obtain a bead of liquid, was found to give consistent results.

(c) To check the penicillin, 4 mm. loopfuls of the solutions of 10 and/or 5 Oxford units per cubic centimeter (1:500 or 1:1000 dilutions, respectively, of 5,000 Oxford units employed therapeutically) should be placed on agar seeded with the Oxford strain of *Staph. aureus*. A growth inhibition zone of 29 ± 1 mm. in diameter with the dilution of 10 Oxford units per cubic centimeter (or 25 ± 1. mm. in diameter with that of 5 Oxford units per cubic centimeter) indicates that the penicillin solution being employed is of suitable concentration.

(d) Place the seeded Petri dish to which the loopfuls of penicillin have been added into the incubator (*right side up*), taking care to see that the bottom of the dish is horizontal in order to insure circular zones of growth inhibition.

C. Examination and record

1. After incubation overnight (from eighteen to twenty-two hours), measure the diameters of the growth inhibition zones to the nearest 0.5 mm. (This may be done by placing the Petri dish on a plate counter and slipping a thin rule graduated in millimeters under it, etching a scale on the plate counter, or merely holding up the plate against a light and measuring the inhibition zone directly by placing a rule on the bottom of the dish.)

Note: If necessary, the probable zone of growth inhibition may be tentatively estimated from eight to ten hours after addition of penicillin to the plates.

2. Record, from Table II, the probable concentrations of penicillin permitting and preventing growth in broth corresponding to the average diameter of the inhibition zones observed.

SUMMARY AND CONCLUSIONS

1. The basis of a technique for evaluating resistance of bacteria to penicillin by determining the zone of growth inhibition when a 4 mm. loopful of penicillin solution (10 or 5 Oxford units per cubic centimeter) in phosphate buffer is placed on the surface of nutrient or blood agar, seeded with a test organism, is developed in detail.

2. A table is presented which shows the relation of the zone of growth inhibition (produced by placing a 4 mm. loopful of a solution containing 10 or 5 Oxford units of penicillin per cubic centimeter on the surface of a seeded agar plate) to the probable concentrations of penicillin which will prevent or permit growth of staphylococci in nutrient broth.

3. The loop growth inhibition zone technique described for determination of bacterial resistance to penicillin is subject to less error, requires no special apparatus and considerably less media and equipment, and is not subject to the hazard of contamination associated with dilution techniques. The resistance can generally be tentatively determined within eight to ten hours after addition of penicillin to seeded plates.

4. When employing a penicillin solution containing 10 Oxford units per cubic centimeter with the technique described, growth inhibition zones of less than 10 mm. in diameter are considered to indicate that the test organisms are resistant; organisms producing growth inhibition zones of from 10 to 15 mm. in diameter are considered to be on the border line; and bacteria which show growth inhibition zones greater than 15 mm. in diameter are considered to be so susceptible as to warrant expectation of a satisfactory response to present methods of penicillin therapy.

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A RAPID METHOD FOR THE IDENTIFICATION OF CERTAIN SULFONAMIDE DRUGS

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THE following method of identification of sulfonamide drugs is based on previous findings[1, 2] that certain of these compounds form crystalline cuprous complexes when heated in an alkaline copper sulfate solution containing a reducing sugar. The crystals are specific and reproducible for an individual

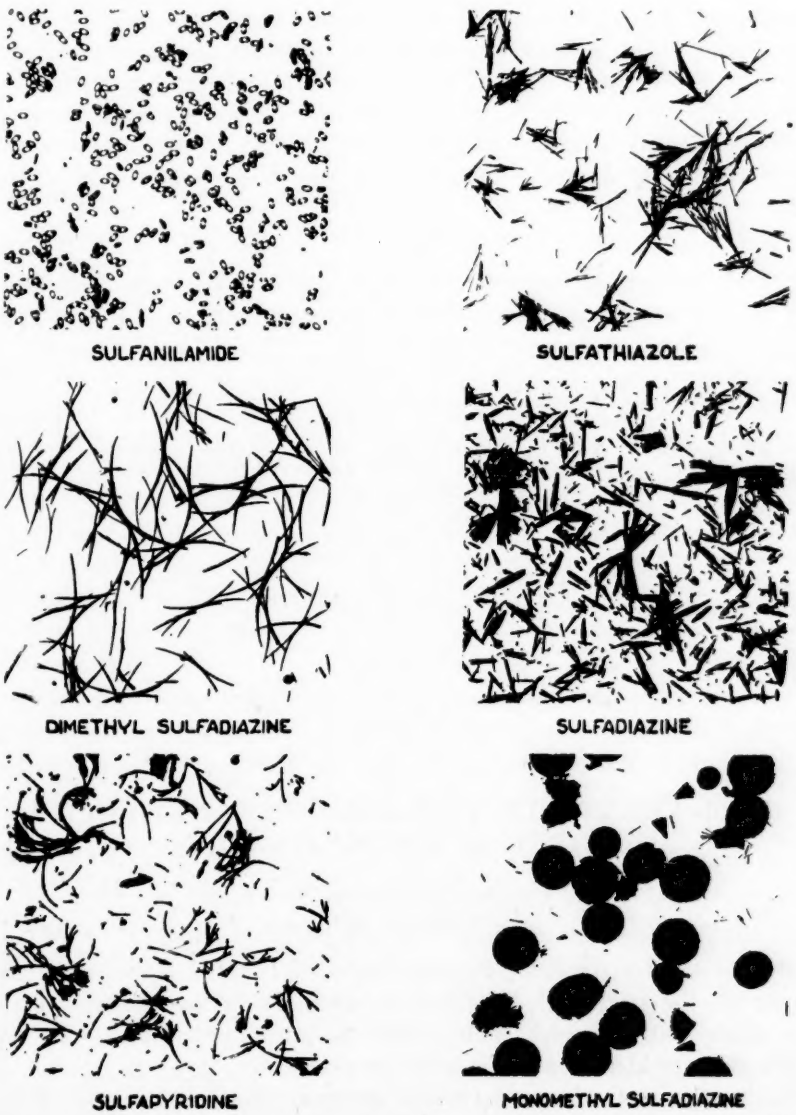

Fig. 1.—Cuprous-copper sulfonamide compounds (×100).

From the Department of Biochemistry, University of Oregon Medical School.
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drug and can be prepared in a few minutes. Identification appears to be more positive by this procedure than by the method of Sample recently reported.[3]

Technique.—Five cubic centimeters of Shaffer Hartman reagent,* 5 c.c. of 100 mg. per cent glucose solution, and about 30 mg. of the sulfonamide drug are placed in a test tube 1 by 8 inches. After heating the mixture for five minutes in a boiling water bath, the crystals formed are examined microscopically. Small amounts of cuprous oxide may form with certain of the drugs, but this does not interfere with the crystal examination.

A description of the crystals is avoided since their preparation is simple, and any individual desiring to use this method of identification would undoubtedly prepare a series of the compounds to become familiar with their microscopic appearance. The structures vary greatly from drug to drug, but under similar conditions of alkalinity and concentration any one drug shows a remarkably constant crystal structure.

Sulfaguanidine and N^4 substituted sulfonamides do not react with cuprous copper under these conditions; consequently their identification cannot be carried out by this method. Sulfanilamide, sulfathiazole, sulfapyridine, sulfadiazine, mono-methyl sulfadiazine (sulfamerazine) and di-methyl sulfadiazine (sulfamethazine) are readily and positively identified by the procedure outlined.

Attempts to apply the method to urine containing sulfonamide drugs have been unsuccessful.

SUMMARY

A rapid method for the positive identification of sulfanilamide, sulfathiazole, sulfapyridine, sulfadiazine, sulfamerazine, and sulfamethazine is reported. This is done by microscopic examination of the crystalline cuprous complexes of these compounds.

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*This is used for simplicity. Other solutions of comparable strength with respect to alkalinity and cupric ion are satisfactory.

IMPROVEMENTS IN THE FLUORESCENT METHOD FOR DETECTING TUBERCLE BACILLI

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THE fluorescent method for detecting tubercle bacilli has several advantages. However, the published directions seem to give slides of varying quality. Bodies, other than tubercle bacilli, retain the auramine stain. These artefacts interfere with the identification of the tubercle bacilli.

The present method was devised to overcome these difficulties. This is accomplished by blending the sputum with a neutral medium. The blending

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tends to separate the bacilli from the artefacts and make their location easy. A good blending agent is a water solution of methyl cellulose. This is sold under the trade name Methocel.* The sales literature describes the properties of this material. The various types of methocel have different thickening powers. The highest viscosity type 4,000 cps seems to be the most useful for the present purpose.

The quality of the slides seems to be improved by treating the sputum with a water solution of pancreatic enzymes. The addition of dried bile to the enzyme solution may also be helpful in reducing the artefacts. The enzyme may act to reduce the artefacts because the latter are fatty substances that react with lipase, or the enzyme may alter the structure of the sputum so that better blending results. Good slides can be prepared without the enzyme treatment, and users may decide to simplify the method by its omission.

Complete directions will be given, which should allow ready duplication of the results.

REAGENTS

Enzyme Solution.—Mix 10 c.c. water, 0.5 Gm. of pancreatin (U. S. P.)† and 0.2 Gm. dried ox bile.‡ Allow to stand fifteen minutes and filter with No. 2 Whatman paper.

Blending Solution.—Mix 25 c.c. water and 0.5 Gm. of methocel type 4,000 cps. Boil gently for fifteen minutes and store in a refrigerator until a clear solution is obtained.

Auramine Stain.—Dissolve 0.1 Gm. of auramine 0§ in 10 c.c. ethyl alcohol, then add 90 c.c. water.

Destaining Solution.—Add 2 c.c. concentrated HCl to 98 c.c. water.

PROCEDURE

Step 1.—Select a small sample of sputum, add an equal volume of enzyme solution, and allow to stand thirty minutes.

Step 2.—Add an estimated 5 volumes of blending solution to the treated sputum and stir vigorously until a smooth mixture is obtained. Spread on the slide in a film of medium thickness, covering the entire slide except for one end. Air bubbles trapped in the smear disappear in the processing. Proceed to step 3 without drying.

Step 3.—Immerse the slide in auramine stain for five minutes.

Step 4.—It is very important to remove all excess stain. The following method should be carefully followed, unless a better one can be devised.

(a) Immerse the stained slide for five minutes in water heated to about 40° C.

(b) Remove the slide, thoroughly stir the material on it with a glass rod, and allow the slide to dry partially for five minutes.

This washing and stirring is repeated until the slide is colorless, except for a slight yellow haze. The slides usually clear on the sixth washing. This step ends with a final stirring and drying for five minutes.

Step 5.—Immerse the slide for exactly one minute in the destaining solution. Remove the slide, stir the material, and allow the slide to dry for five minutes; then rinse the slide in cold water, dry, and examine.

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*Dow Chemical Co., Midland, Mich.
†Eli Lilly and Company, Indianapolis, Ind.
‡Difco Laboratories, Inc., Detroit, Mich.
§National Aniline Division, Allied Chemical & Dye Corporation, New York, N. Y.

The usual method specifies 3 per cent phenol in the auramine stain, although Richards and co-workers[1] have obtained satisfactory results with 2 per cent phenol. The presence of these amounts of phenol modifies the methocel and causes partial disintegration of the smear. A phenol content of 1 per cent can be tolerated, but it seems better to omit it entirely. A considerable number of slides have been stained with stain containing no phenol and also 1 per cent phenol. The results seem to be satisfactory.

This general technique might be adapted to the identification of other organisms by fluorescent microscopy. Pringsheim and Vogel[2] list several "fluorochroms" which might be used as staining agents.

EXAMINATION OF THE SLIDES

Directions for converting an ordinary microscope for fluoroscent work have already been published.[3] The CH4 mercury lamp works well, and a less efficient light source probably will not be satisfactory.

Slides should first be prepared from several positive sputa to show the various sizes and groups of tubercle bacilli. The bacilli can be located with the usual N. A. .66 objective and identified under oil immersion, using clear mineral oil. They appear very well with the usual N. A. 1.25 objective.

In case large numbers of slides must be examined, other objectives can be tried which sacrifice some resolution for convenience of use. The following objective was tested and seems to have advantages: 40 × 4.3 mm., N. A. 1.00 fluorite oil immersion.*

The magnification is rather low, but the good resolution, depth of focus, and area of field are attractive features. Dry objectives with N. A. .85 or .95 may also be adequate.

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*Bausch & Lomb Optical Co., Rochester, N. Y.

A SIMPLE COUNTER-RECORDER OF DROPS OR OTHER EVENTS

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MANY drop recorders have been described. Emphasis has usually been on modifications at the dropping end, with an ordinary signal magnet used for kymographic recording. To minimize the labor of counting recorded drops or other events, various adaptations of Fleisch's Ordinatschreiber have been used, but such special equipment as the latter is not readily available in many laboratories. In seeking a simple, easily assembled adding recorder, we resorted to the device illustrated in Fig. 1. It has been found most convenient and serviceable during about two years of use.*

From the Research Laboratories, Parke, Davis & Co.
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*We are indebted to Mr. R. W. Thomas for the major part of this trial.

As we have used it, the recorder replaces the ordinary signal magnet. It is operated by the output of a 120 volt D.C. electronic relay activated by electrolytic closure of the input circuit through a drop falling between suitable platinum electrodes. The basic unit of the recorder is a commercially

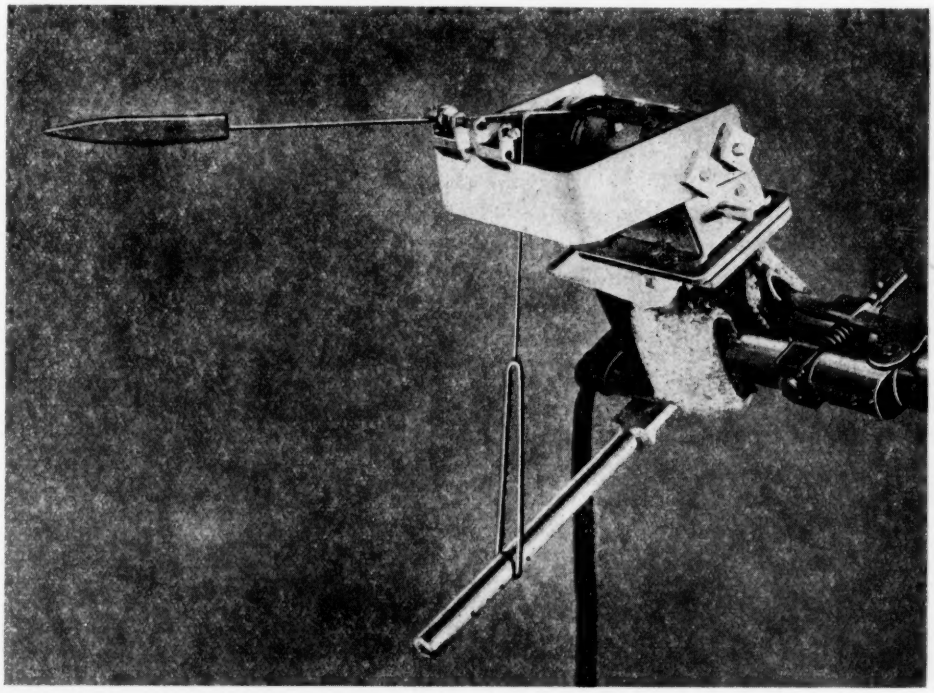

Fig. 1.—The recorder, mounted in an ordinary burette clamp, in operating position. Ten-event lever in place, with fulcrum provided for the hundred-event lever.

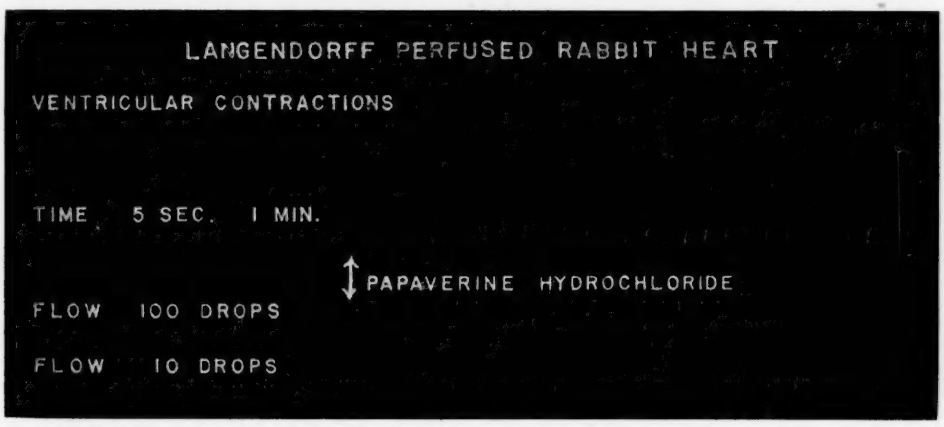

Fig. 2.—Illustration of simultaneous ten- and hundred-event recordings (last and next to last records). Note individual and tenth event signals in the one and individual and hundredth event signals in the other.

available electromagnetic counter* built for 120 volts D.C.† A U frame is screwed to the ends of the counter case for mounting one, two, or three compact heart-lever bearings. Steel wire recording levers are shaped to operate at these fulcra to record vertically with respect to one another and to ride at

*Gorrell and Gorrell High Speed Electric Counter, by Gorrell and Gorrell, Chicago Heights, Illinois.

†Other current capacities in relay and counter could be used, according to the current source most conveniently available.

their inner ends on the peripheries of respective counting wheels. As much of the plastic window of the counter is removed as is necessary to admit the levers. The riding end of each lever is bent over axially on its wheel and then downward a small distance and forward along the inner surface of the wheel in such manner as to be lifted slightly by the double-tooth projecting from this surface. The tooth is an integral part of the counter, serving to turn the next adjacent wheel one unit per revolution of the first wheel. Slight tension of the levers on their respective wheels is maintained by adjustable rubber bands or springs. In the instrument illustrated only one lever is in place.

In use (Fig. 2), the vibration of electromagnetic activation conveniently serves to record individual events (for example, drops) through each lever. In addition, each tenth event is marked off positively by the first lever, each hundredth by the second, and each thousandth by the third. In our current use of the recorder, the ten-event integration by the first lever suffices. Such marking off of tens, hundreds, or thousands of events greatly facilitates quick tabulation of the recorded data and would enable the use of very slow kymograph speeds and/or convenient recording of high frequency events. Any kind of event capable of electrical signaling, other than falling drops, can be recorded in this manner provided that the frequency does not exceed the relay's or counter's maximum (for example, several hundred per minute claimed by the maker of the counter used).

An electric counter principle has been used previously in integrated kymographic recording but apparently by intermediation of electrical circuits beyond the counter instead of simple mechanical means.[1, 2]

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BOOK REVIEWS

Bronchial Asthma. By *Leon Unger,* B.S., M.D., F.A.C.P., Assistant Professor, Department of Medicine, Northwestern University Medical School, Chicago. Charles C. Thomas, Springfield, Ill. Price $9.00. Cloth with 730 pages and 126 figures.

Bronchial Asthma by Leon Unger is a good book. Wartime publishing problems are not apparent in its printing, paper, binding, or proofreading. There are 731 pages in Parts I, II, and III and in the introduction, preface, and index.

The introduction states that theoretical considerations will be kept at a minimum. The result is a practical, conservative, conventional presentation of the author's opinions plus a comprehensive review of the literature of asthma.

Part I, the clinical section, has seventeen chapters in 625 pages. The first chapter, The Language of Allergy, is a historical glossary. The second chapter, an excellent history of bronchial asthma, has several references not found in the usual books on allergy. The five chapters on etiology are detailed and well organized. The chapter on pathology summarizes the relevant literature and presents the author's studies in considerable detail. Other chapters on diagnosis, skin testing, and specific and nonspecific treatment are concise but comprehensive.

Part II, the laboratory section, reviews in twenty pages the preparation of extracts, pollen, and mold spore counting and the technique of the leucopenic index.

Part III has sixty-seven pages of details which the allergist uses to instruct his patients in the effective avoidance of food and inhalant allergens.

The bibliographies at the end of each chapter are worth the price of the book. The chapter summaries are concise and informative.

The chapter on Military Aspects of Asthma was timely when the book was printed. With revision, it should be of continued value to allergists in other countries.

No textbook is perfect. This one is no exception. There is little doubt that another edition will be in demand. In the new edition, brevity and simplicity could be furthered by a careful sifting of redundant and irrelevant matter, for example, by not discussing treatment, allergen avoidance, and constitutional reactions under etiology, by fewer summaries of papers which have essentially the same message, by materially shortening the sections on drugs and hormones in which most of the references are to other allergic manifestations than asthma, by omitting the chapter on other manifestations of allergy.

Every allergist who reads the book will find something with which to disagree, but allergists are "rugged individualists" who like to disagree.

It should be repeated that this is a practical, conservative, conventional, comprehensive survey of bronchial asthma.

OSCAR SWINEFORD, JR.

Dietotherapy. Clinical Application of Modern Nutrition. Edited by *Michael G. Wohl,* M.D., Associate Professor of Medicine, Temple University School of Medicine; Chairman, Advisory Committee on Nutrition, Philadelphia Department of Public Health. W. B. Saunders Company, Philadelphia. Price $10.00. Cloth with 1,029 pages and 93 illustrations.

It is difficult to review a book in which each chapter is a review written by separate and individual authorities each in his special field. The editor's claim that each of his contributors is a recognized investigator must be granted, and his hope also that "this book will provide the practicing physician with current advances and practical applications" is well justified.

He is to be congratulated on getting experienced and able clinicians to write in a practical manner on such fundamental topics as water metabolism, trace elements in nutrition, and even on tracer substances.

The contributions on the vitamins seem well suited to human consumption although written in most instances by authorities in agricultural colleges and laboratories of biochemistry. This reviewer finds it rather stimulating to be told what the "clinical possibilities" of a B vitamin may be long before the vitamin has been tried clinically.

The purpose of the book as set forth in the foreword might have been better accomplished by some editing to bring the nutritionists and the dietotherapists into closer agreement. For instance, the foreword written in 1942 in part by a committee of the National Research Council states that the physician may do with a "biomicroscope" in examination of patients what the pathologist has for the methods of pathology by his microscope. However, in 1945 there seems to be no contributing author who even mentions the biomicroscope. Likewise, the foreword's criticisms of customary corrective diets used in gastroenterology, as in peptic ulcer, seem unheeded in the section dealing with ulcer. Perhaps this discrepancy is a fault of medical practice rather than one of editing a book.

On the whole, the nutritional theorists have not run away with this book. It is a good text on dietotherapy, even though it frequently calls for "easily digested" food without ever explaining in a most thorough chapter on digestion just what "easy digestion" is.

This shows that dietotherapy is still a useful art, even if outstripped by nutritionists. The book is, in the opinion of the reviewer, as good a junction of theoretical nutrition with practical dietetics as can now be made.

M. A. BLANKENHORN, M.D.

Fundamentals of Pharmacology. By *Clinton H. Thienes,* M.D., Ph.D., Professor of Pharmacology, University of Southern California School of Medicine, Attending Pathologist (Toxicology), Los Angeles County Hospital. Paul B. Hoeber, Inc., New York. Price $5.75. Cloth with 497 pages.

This volume is one of a series of texts which are being edited by Dr. Fred C. Zapffe as secretary of the Association of American Medical Colleges. The object in writing these books is to present authoritatively the basic foundation and to omit nonessentials in the subjects covered. The aim is to create books the student will have time to read in spite of the great pressure which is put upon him from all directions. This is a splendid idea and the need for progress in this direction is long since overdue.

The present volume covers the field of pharmacology in twelve sections as follows: (1) Stimulants of the Central Nervous System; (2) Depressants of the Central Nervous System; (3) Drugs Acting on the Peripheral Nervous System; (4) Drugs Acting on Muscles; (5) Diuretics; (6) Antiparasitic Drugs; (7) Hormones, Special Minerals, and Tissue Extracts; (8) Vitamines; (9) Drugs Used Because of Local Action on Body Surfaces; (10) Chemical Diagnostic Agents; (11) Actions of Drugs on Cells; (12) Pharmacy and Prescription Writing.

This is an admirable small text. In most instances it presents more of the pharmacology of any given drug than the average medical student will be able to learn in the time, and with the opportunities, at his disposal. No attempt is made to cover in detail a vast array of drugs, but practically all of those in extensive use at present are discussed, including a series of the sulfa drugs, demerol, and penicillin. The subject of Prescription Writing is covered in eighteen pages, English being used in preference to Latin. A useful bibliography follows each general section throughout the book.

In general, this will be a very helpful book for medical students, especially for review purposes.

D. E. J.